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*From portrait by Seymour Thomas*

SIR WILLIAM OSLER

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On several occasions, some of which are mentioned herein, Dr Osler counseled his students and his readers to review and to weigh periodically the accumulated knowledge of disease, to find the way of future advances. One hundred years after his birth, this issue of the Bulletin of the Johns Hopkins Hospital, in the founding of which he took a special interest, contains summaries of the progress and the present status of four diseases to which Dr Osler made particular and important contributions.

THE EDITORS

# INFECTIOUS (SO-CALLED ULCERATIVE) ENDOCARDITIS

BY WILLIAM OSIER, M D , M R C P , LOND ,

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UNDER the terms *diphtheritic, ulcerative, malignant, septic, or infectious* endocarditis, *arterial pyæmia, mycosis endocardii*, physicians now recognize one of the most formidable of cardiac affections, characterized by a peculiar morbid process on the valves, blood contaminations, constitutional symptoms of the typhoid or pyæmic types, and usually associated with multiple emboli

It is only within the past few years that the subject has received due attention in the text-books, indeed, in some it is barely touched upon, and even in recent manuals on heart disease the account is not very satisfactory

From the number of reported cases in French and German journals, and from the interest which the disease has excited in these countries, we might suppose it to be more common there than in England or America. A considerable number of reports, however, occur in the "Transactions of the Pathological Society of London" and in the British journals. In the leading American periodicals there are very few references, but cases have

# SIR WILLIAM OSLER AND BACTERIAL ENDOCARDITIS

WARFIELD T LONGCOPE

It was in 1875 that Dr Drake retired from the Professorship of "The Institutes of Medicine" at McGill University. Osler had hoped to obtain the position of demonstrator in this department, but he was now given the position of lecturer, though he was only 25 years of age, and at the completion of the semester was appointed Professor of "The Institutes of Medicine" to succeed Dr Drake. He was thus made responsible for the rather dreary task of lecturing on Physiology and Histology.

At this time, however, there was an epidemic of small pox in Montreal and Osler spent much time in the wards of the small pox hospital. His interest in pathology led to his appointment the following year, in 1876, to the status of Pathologist to Montreal General Hospital, and two years later at the age of 28 to the important post of Physician to the Montreal General Hospital. As pathologist, during this period, he made many brief reports including those on Aneurysm, endocarditis and diseases of the heart. It was, therefore, early in his career that his intense and life-long interest arose in these subjects.

It was not until 1881, however, that his first contributions to the problem of infectious endocarditis appeared, one in the Archives of Medicine, and a second in the Transactions of the International Medical Congress in London.

In order to trace the observations that culminated eventually in the recognition of a particular form of endocarditis which is now usually designated as Subacute Bacterial Endocarditis, it is necessary to quote "in extenso" from one after another of his articles. In doing so it will be desirable to call attention to the particular observations that bear upon the development of a knowledge of this disease. It must be remembered that when these early articles appeared, bacteriology was in its infancy and Koch's discoveries, with his monumental work on the tubercle bacillus, had not been published.

Abstracts from Osler's paper on infectious endocarditis follow

## INFECTIOUS (SO-CALLED ULCERATIVE) ENDOCARDITIS (1)

BY WILLIAM OSLER, M D , M R C P , LOND

*Professor of the Institutes of Medicine, McGill University, Physician and Pathologist to the General Hospital, Montreal*

(Arch Med 1881, 5 44)

"Under the terms *diphtheritic, ulcerative, malignant, septic, or infectious* endocarditis, *arterial pyaemia, mycosis endocardii*, physicians now recognize one of the most formidable of cardiac affections, characterized by a peculiar morbid process on the valves, blood contaminations, constitutional symptoms of the typhoid or pyaemic types, and usually associated with multiple emboli

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With regard to the nomenclature, I think the terms *infectious* and *septic*, as given by Jaccoud,|| better than the others. Against the name *ulcerative* is the fact that there may be no actual ulceration on the valves, and there may be, on the other hand, endocardial losses of substance without the special constitutional disturbances by which the disease is characterized. The term *diphtheritic* is good, in so far as it expresses a resemblance in the histological features of the valvular disease to that of true diphtheritic exudation, but this is scarcely sufficient ground for its use, and it is, in a way, misleading, indicating a relation between diphtheria and the disease, which is not known to exist. The name *mycosis endocardii* certainly expresses a striking feature of the local process, but with our present imperfect knowledge of the relation of the micrococci colonies to the disease, such a designation is, to say the least, premature. On the other hand, the term *infectious* presupposes no special view as to the nature of the local process, and at the same time indicates, as Jaccoud says, a constant and exclusive character of the disease

\* *Boston Med and Surg Journal*, Nov 15, 1877

† *Philadelphia Medical and Surgical Reporter*, 1874

‡ *American Journal of Medical Sciences*, 1871

§ "Transactions of the College of Physicians of Philadelphia," 1879

¶ *New York Med Record*, 1880

|| *Pathologie Interne*, tome 1, and *Nouveau Dictionnaire*, tome III

It would appear that, clinically, three classes of cases are included in the disease known as ulcerative endocarditis, and I think it important that a distinction should be made between them. We have

1 Those cases in which the disease appears without any obvious cause, either spontaneously or in connection with rheumatism or some other affection. The term *infectious* might be applied to this class. It is the *arterial pyæmia* of Wilks, the primary ulcerative endocarditis of some authors.

2 Those in which the endocardial disease is secondary to some inflammatory focus—acute necrosis, puerperal endometritis, etc. To these the term *septic* might be applied.

3 In certain cases of chronic valvular disease an acute endocardial process may be engrafted (recurrent endocarditis), presenting anatomical features similar to the infectious form, but not characterized by the same clinical picture, the patients dying with the symptoms of chronic heart disease.

The following paper embodies my experience of this disease. The chief points to which I wish to call attention, and which are illustrated by the cases, are

1 That the majority of cases of infectious endocarditis occur independently of rheumatism.

2 To the frequency with which infectious endocarditis is associated with pneumonia.

3 The production of acute multiple aneurisms of the aorta in the disease.

4 To certain histological features in the endocardial vegetations, and particularly to a remarkable fungoid growth met with in one of the cases."

After the introduction there follow histories and autopsy notes on seven cases of "Infectious Endocarditis." Cases 1, 2, 3, 4 and 6 are clearly instances of pneumonia complicated by an acute and rapidly fatal endocarditis.

The fifth case is also considered to be one of pneumonia, but the autopsy disclosed certain interesting features. The vegetations involved, not only the aortic valves but the wall of the left ventricle below them. In addition to this the note is made that the aortic valves were bicuspid. Though Osler interpreted the pulmonary lesions as those of a resolving pneumonia, the general clinical and pathological picture with the plaque of vegetations plastered upon the wall of the ventricle below the aortic valves, as well as the multiple aneurisms of the ascending aorta which contained vegetations are all suggestive of an extensive subacute bacterial endocarditis. There might be some question, from the description given of the aortic valves whether this condition was of congenital origin, but the observation is noteworthy in view of the prominence given many years later to the occurrence

of bacterial endocarditis upon bicuspid aortic valves For these reasons two paragraphs from the discussion of this case are quoted

"The presence of multiple aneurisms of the aorta in Case 5 is deserving of comment, as I have not been able to find any similar observation in the literature of either ulcerative endocarditis or of aneurism

The man had evidently been the subject of that peculiar congenital malformation of the aortic semilunar valves which results in the fusion of two segments In this condition they are very liable to be the seat of a sclerotic endocarditis which terminates in incompetency, and I have met with two other cases in which the united curtains, when in this state, were the seat of extensive ulcerative endocarditis \* The cardiac affection was evidently of old standing, and in February, 1879, a year and four months before his death, hypertrophy, a double murmur and a thrill were noted "

Case 7 might well be considered as a possible instance of subacute or chronic bacterial endocarditis and is therefore quoted below in full

"CASE 7 — *Sclerotic endocarditis of aortic valves, with incompetency, recent vegetations (ulcerative endocarditis)*

Annie M L, aged 40, admitted Nov 23d under Dr Ross

No history of acute rheumatism For five or six years has suffered with shortness of breath on exertion For the past year health has been failing, and she has had a troublesome cough For three months has been confined to bed, dropsy has gradually come on, and for three weeks past spitting of blood Her condition on admission was that of a patient in the advanced stage of obstructive heart disease, —great dropsy of legs, right hydrothorax, dyspnoea, lividity, cough, and bloody expectoration A double aortic murmur was determined She only lived for a little over two days after entering the hospital The temperature was normal

At autopsy *heart* was large, chambers full of dark clots Mitral valves healthy Left ventricle dilated and hypertrophied The aortic orifice was blocked with vegetations, and when slit open the valves were found much diseased, all the curtains were thickened, curled at the edges and foreshortened On the ventricular faces were large grayish-yellow vegetations, closely adherent, but friable and roughened on the surface In one mass the deposition of salts of lime had taken place on the outer part Large patches of apoplexy in the *lungs* No infarcts in *spleen* or *kidneys*, which were large and indurated

This is an illustration of the third class, and perhaps such instances furnish the large proportion of the cases which go under the heading of ulcerative endocarditis "

The remainder of this article is devoted to a detailed description of the gross and microscopic appearance of the vegetations, together with

\* *Virchow's Archiv* lxi, 1875

a brief discussion as to the significance of the "micrococcus balls" which he sees and pictures in what appear to us today to be quite crude drawings. They are described as follows:

"In the larger outgrowths the chief mass is composed of a nucleated fibrillar tissue, while in the superficial parts there are fibrinous lamination and numerous micrococci colonies. Capillary blood-vessels penetrate the deeper parts of the large masses, and along many there is a deposition of brown-red pigment. In some sections large micrococcus balls were met with 4 or 5 mm from the surface.

The most remarkable structures in this specimen are the rounded bodies represented in Figs 1 to 6, and which have been spoken of above as micrococcus balls. They vary very greatly in size, the majority of those in the specimen from which Fig 1 was taken measured from 0.15 to 0.375 mm. Many are not more than 0.075 mm, while at Fig 6 one is shown which measured 1500 by 1125  $\mu$ m. In places they occur in hundreds, closely set together, and often very small, as at Fig 4. The outlines are sharply defined, but it is not certain whether they possess a definite membranous investment. They contain minute refractile granular spherules, which behave with reagents like micrococci. In some of the larger balls, as shown in Fig 6, secondary ones can be seen.

I am not prepared at present to discuss the nature and affinities of these structures, but hope to do so on another occasion, when I shall enter more fully into the histology of the primary and secondary lesions of this disease."

He then proceeds to discuss the etiology of "Infectious Endocarditis" and points out first, the fact that primary infectious endocarditis in the majority of cases does not occur in connection with acute rheumatism, as was almost universally stated to be the case, secondly, the frequency with which this disease occurs with pneumonia, and thirdly, a point of clinical interest, namely, the occurrence of meningitis in three of his seven cases which he has analyzed.

The paper concludes with an account of the contemporary view as to the importance of micrococci as exciting agents together with his own opinion of their significance. In relation to our present knowledge of the subject it is interesting to quote the final paragraphs in full:

"With regard to the intimate pathology of this disease, it is assumed by most recent writers to be a mycosis, i.e., to be dependent upon the growth and propagation of lowly fungi on the valves with a consequent blood contamination. Certainly the minute bodies found in the endocardial vegetations correspond in their chemical and microscopical relations to micrococci. They are motionless, highly refractile spherules, less than a micro-millimetre in diameter, arranged in groups or colonies without any perceptible stroma. Acids, alkalis, ether and chloroform



have no effect upon them. These characters are supposed to afford satisfactory means for distinguishing them from granular detritus of an albuminous or fatty nature. Most writers have accepted the view that these bodies are fungoid in nature. Heller,\* however, criticizes strongly the prevailing conceptions with regard to micrococci, and thinks that there are scarcely any micro-chemical agents or physical signs by which they can be distinguished from fatty detritus. He recommends soaking the tissue in 10 per-cent potash solution and then in iodine solution, 1 in 10 of spirit, which tints monads yellowish-brown, but is inert on fat granules. Sections of the vegetations in these cases, treated in this way, show the colonies stained of a brownish-yellow color.

Apart from any micro-chemical tests there are peculiarities about these masses which we do not see in any form of fatty degeneration, as the uniformity in size of the granules and their collection into large groups.

The question of the relation of the micrococci to the disease presents many difficulties, and we are probably not yet in a position to give a final answer to the problem. Klebs, and most German writers on the subject, give an unhesitating assent to the parasitic theory and suppose the micrococci to gain access either through the gastro-intestinal or respiratory systems, and they believe them to constitute the actual *materies morbi*. According to Koster† and Klebs‡ not only are these fungi present in the so-called ulcerative form, but they also exist in, and cause the development of, the ordinary warty or bead-like vegetations so frequently met with in the valves. Within the past few months I have examined four specimens of this variety of endocardial vegetation, and have been able to determine in each instance the presence of micrococci, not, it is true, in the same luxuriance, or arranged in definite colonies, but still sufficiently distinctive. In one case of mitral stenosis a fresh vegetation, when teased, showed many closely-packed spherules, some of which were, as Klebs has remarked with reference to the micrococci in this variety, larger than those met with in the ulcerative form. I was greatly struck with the resemblance which certain of these bodies, in this instance, bore to the individual elements of Schultze's granule-masses—those peculiar granular clumps common in blood of some animals and of impoverished persons. These structures are usually regarded as the *debris* of colorless blood corpuscles, but I have shown§ that they are aggregations of discoid bodies, probably living organisms of the nature of which we are still ignorant. They do not exist in the form of masses in the blood, but as isolated particles which might readily become adherent to the fresh endocardial outgrowths. I merely mention this as a point worthy of future investigation.

It matters little how the micrococci get to the valves, whether by embolism of the small vessels, as Koster supposes, or by deposition on the surface, as Klebs thinks, the question is: Are they responsible by their growth for the peculiar course

\* *Virchow's Archiv* lxi, 1875

† *Virchow's Archiv*, Bd lxxii

‡ *Archiv für Exper Pathol u Pharmacol*, Bd, ix

§ Proceedings of the Royal Society, 1873

and malignancy of cases of infectious endocarditis, primary or secondary? The facts of their occurrence in the verrucose form, which may not be accompanied by any symptoms, and of their abundance in the recurrent endocarditis, which attacks old sclerotic valves, are, I think, opposed to this view, for if they act as a septic poison in the one case, why should they not do so in the other? The micrococci do not appear to infest the blood in any numbers, so that they must be supposed to distil some subtle poison, "such soon-speeding gear as will disperse itself through all the veins" and profoundly disturb nutrition. The occurrence, however, of fatal septic cases, closely allied to, or identical with those in which a bacteric endocarditis is found, but in which no micrococci can be detected, either in the local process or in the blood, teaches us that the same poison may exist without the intervention of bacteria, the presence of which in any case may be only a partial phenomenon in a general infective process."

The second paper published in 1881 appears to be a condensed version of the large article which has just been reviewed, and it is only desirable to quote a few paragraphs. The first outlines the scope of the communication.

The second paragraph seems to forecast a group of cases, namely (3), the nature of which becomes clearer as Osler's studies proceed.

### ON SOME POINTS IN THE ETIOLOGY AND PATHOLOGY OF ULCERATIVE ENDOCARDITIS (2)

DR WILLIAM OSLER, MONTREAL

(Trans Internat Med Cong, London, 1881, 1 pp 341-346)

"Ulcerative, infectious, or diphtheritic endocarditis is an affection of unusual interest to the profession, both on account of the serious nature of the malady which it excites and of the illustration which it offers of many points in the pathology of infective processes.

Ulceration, loss of substance, on the endocardium occurs under a variety of conditions. Clinically we should, I think, recognise three classes of cases. *First*, those in which the disease appears without any obvious cause, either spontaneously or in connection with rheumatism or some other affection, as pneumonia, chorea, &c. These cases present a remarkable set of symptoms, very variable in character, but of which two chief types have been recognised—the typhoid and the pyæmic. This is the preliminary form of some writers. *Second*, those cases which arise during the existence of some local inflammatory process, as puerperal endometritis, acute necrosis of bone, &c, and in which the endocarditis is usually regarded as part of a pyæmic state and secondary to the local disease. And, *third*, the cases of ulcerative affection engrafted upon valves the subject of chronic sclerotic changes. In this latter variety no special symptoms necessarily accompany the process, the patients are usually in the last stage of chronic valvular disease.

I propose to consider briefly in the following paper some of the conditions under which the disease arises, some points in the morbid anatomy, and, lastly, make a few remarks on the supposed relation of micrococci to the disease "

During the next few years, according to Cushing (3), Osler was working on the subject of endocarditis, collecting cases, doing autopsies, and gathering a large amount of material together. In 1883 he was made fellow of the Royal College of Physicians, and after a summer in Europe in 1884, he accepted an offer from the University of Pennsylvania to become Professor of Clinical Medicine, a post made vacant by the promotion of Dr William Pepper to the senior Professorship of Medicine.

He was invited, about this time, to give the Gulstonian Lectures before the Royal College of Physicians, and he chose as his subject "Malignant Endocarditis." These three lectures delivered on Feb 26th, March 3rd, and March 5th, 1885, have survived since their publication as a classical description of the disease.

The first lecture opened with a short general account of the knowledge of this condition as it then existed, and a statement that these lectures were based upon a review of over 200 cases that he had collected from the literature, supported by his own observations upon a large series of cases that he had studied in the wards of the Montreal General Hospital or at autopsy. The introduction to these lectures was as follows:

THE GULSTONIAN LECTURES,  
ON  
MALIGNANT ENDOCARDITIS (4)

Delivered at the Royal College of Physicians of London, March, 1885

By WILLIAM OSLER, M D

*Professor of Clinical Medicine at the University of Pennsylvania, Philadelphia*

(*British Med J* 1885, 1 467, 522, 577)

LECTURE I

"MR PRESIDENT AND GENTLEMEN,—It is of use, from time to time, to take stock, so to speak, of our knowledge of a particular disease, to see exactly where we stand in regard to it, to inquire to what conclusions the accumulated facts seem to point, and to ascertain in what direction we may look for fruitful investigations

in the future With your permission, sir, I propose to do this in the case of that most interesting disease generally known as ulcerative endocarditis, a disease the phenomena of which were first clearly explained by the late Dr Karkes, from whose investigations in 1851-52 we date our accurate knowledge of the affection Some of those who listen to me today can doubtless recall, and recall with pleasure, the Gulstonian Lectures of 1851, in which Dr Ormerod dealt so fully and so ably with valvular affections of the heart, but a reference to them will show how much the past twenty-five years have done to widen our view of cardiac disease, more particularly in regard to the effects of emboli, and the association of valvular inflammation with grave constitutional disorder, and the probable connection of the disease with the presence of micro-organisms By the labours of Drs Ogle, Wilks, Simpson, Moxon, Bristowe, and others in this country, of Charcot, Vulpian, and Lancereaux in France, and of Virchow and a host of observers in Germany, a large amount of material has been accumulated, and we may assume that the etiological, clinical, and anatomical characters of the disease have been fairly well ascertained, and that we have got about as far towards a full knowledge of the affection as the ordinary means at our disposal will permit The inquiry now enters upon another stage, and it remains for experimental investigation to determine, if possible, the relation of the endocarditis to those diseases with which it is most frequently associated This being the case, the present time has seemed to me a favourable opportunity to summarize our knowledge to date, and, for this purpose, I have reviewed the records of over two hundred cases, which, from the description of the symptoms and lesions, were evidently of the type of malignant endocarditis, and these, with the considerable experience I have had at the General Hospital at Montreal, may perhaps enable me to give a somewhat more comprehensive account, in some respects, than has yet been attempted "

He then points out the difficulties of classification and nomenclature and finally defines the types of malignant endocarditis as follows

"Malignant endocarditis occurs under the following conditions 1, as a primary disease of the lining membrane of the heart or its valves, either attacking persons in previous good health, or more often attacking the debilitated and dissipated, or those with old valve-lesions, 2, as a secondary affection in connection with many diseases, particularly rheumatic fever, pneumonia, scarlet fever, diphtheria, ague, etc , 3, as an associated condition in septic processes, traumatic or puerperal We shall discuss first the anatomical characters, then the clinical features, and lastly the etiological and pathological relations "

He then goes on to describe the gross pathological and histological characteristics of the lesion

The following paragraph concerning the relationship of micrococci to these lesions is, today, of some interest for he states

"The micrococci are constant elements in the vegetations. All granules of an uniform size met with in the sections are not micro-organisms, nor, indeed, are all which stain by some methods recommended for the detection of these bodies. By far the most satisfactory method is that of Gramm (*Fortschritte der Medizin*, Band 1, Berlin), in which the section, after staining in gentian-violet, is transferred for a few minutes to a dilute solution of iodine and iodide of potassium, and then to the alcohol, when it is found that the colour has been extracted from all tissue-elements and nuclei, leaving only the micro-organisms stained. They vary a good deal in number and arrangement, and may be scattered singly in the granular substance or arranged in groups. They are usually very numerous at the deeper part of the vegetations, just where the structureless maternal joins the granulation-tissue, and they may penetrate deeply into the substance of the valve. Sometimes the smaller vegetations seem made up exclusively of them. Several of my specimens appear to confirm the view of Klebs (*Archiv für Experiment Pathologie*, Band vi), that the micrococci lodge first on the endocardium, and penetrate into the substance, often as distinct columns. In their immediate vicinity, there is a zone of necrosis, and beyond this an accumulation of leucocytes and signs of reactive inflammation. The micro-organisms found in connection with the malignant endocarditis are not all of the same kind. Klebs distinguishes two forms, one met with in septic, and the other in rheumatic, cases. In some instances, the micrococci are all arranged in zoogloea-like masses, in others, particularly the septic cases, they are in chaplets. Some present distinct capsules. Small elongated bacilli have also been found, I have seen them in one instance, short stout rods, often joined in pairs."

The frequency with which he found the different valves of the heart affected is of interest and the fact that the endocardium of the ventricles was involved in 33 cases is noteworthy since this finding is so reminiscent of subacute bacterial endocarditis.

"The following figures give an approximate estimate of frequency with which different parts of the heart are affected. The aortic and mitral valves were affected together in 41 cases, the aortic valves alone in 53, the mitral alone in 77, the tricuspid in 19, the pulmonary valves in 15, and the heart-wall in 33. The right heart is rarely affected alone, this occurred in only 9 instances, in 5 of which the tricuspid, and in 4 the pulmonary, valves were involved. The valves are most often attacked along the lines of closure, as in the simple endocarditis, the auricular faces of the mitral flaps and the ventricular surfaces of the aortic cusps suffering most severely. Mural endocarditis is most often seen at the upper part of the septum of the left ventricle, just below the aortic ring, in which situation some of the most extensive and deep cardiac ulcers occur, leading to perforation of the septum. Next in order is the endocardium of the left auricle on the postero-external wall, as noted by Lepine (*Bull de la Soc de Biologie*, 1869)."

Of some significance, also, is the paragraph dealing with the relative

frequency with which the acute process is engrafted on valves that are chronically diseased

"It was Sir James Paget (*Medico Chirurgical Transactions*, vol. xxvii), I think, who first referred to the frequency with which sclerotic and malformed valves are attacked by acute disease. Chronic valvulitis is met with in a large number of cases of malignant endocarditis. The records which I have examined give only a percentage of about twenty-five, but the condition of the valves, except as regards ulceration, was often omitted, and thus represents a very much smaller percentage than actually occurs. In more than three-fourths of the Montreal cases, sclerotic changes were present, and Dr Goodhart found (*Pathological Society's Transactions*, vol. xxxiii), in a series of sixty-nine cases, that sixty-one presented old thickening of the valves. In very many of the cases, the condition of fusion of two of the aortic cusps was present. This abnormality is almost invariably accompanied by sclerotic changes, and to the existence of these is probably due the frequency with which they are attacked by ulceration. In seventeen instances of fusion of two of the aortic cusps of which I have notes, there were ulcerative changes in eight, in two or three of an atheromatous nature."

Later he pays particular attention to the embollic phenomena that often accompany the disease, and refers specifically to the frequency of hemorrhages in the skin.

"In the group of primary cases, the lesions are entirely those of endocarditis, local and general. In the second place, there are the extensive pathological changes due to embolism, and these constitute interesting features in the disease, and may produce a very great variety of lesions in every portion of the body. I do not propose to deal very fully with these, but to call attention only to some special points. The cases may be divided into those without any embolic processes, cases in which the infarcts are simple, not suppurative, those in which there are innumerable suppurative infarcts and cases in which some of the infarcts are simple and some suppurative. It is remarkable how variable these embolic features are. They may be entirely absent in well marked malignant cases. They are not necessarily associated with suppuration, indeed, in a very considerable number of cases, they present the characters of ordinary hemorrhagic infarcts, but in the traumatic and puerperal cases the infarcts are invariably septic. They may be few in number, only one or two perhaps in the spleen or kidney, or they may be in thousands throughout the various organs of the body. When suppurative, micrococci, in my experience, are always present, but the micrococci may exist in the vessels without inducing this change. In severe forms of the disease, haemorrhages are very frequent upon the skin, and on the serous and mucous surfaces. The cutaneous ones will be referred to again in connection with the symptomatology. They appear, in many instances, to be due to the effect of the poison, just as in other

infectious diseases, in others, they are undoubtedly embolic, and a minute necrotic or suppurative centre can sometimes be seen ”

The first lecture ends with a rather detailed description of the pathological appearance of these complications as well as others of a different nature such as meningitis

The second lecture, devoted to a description of the clinical features of the disease, opens with an introductory statement

# THE GULSTONIAN LECTURES, ON MALIGNANT ENDOCARDITIS

Delivered at the Royal College of Physicians of London, March, 1885

By WILLIAM OSLER, M D

*Professor of Clinical Medicine at the University of Pennsylvania, Philadelphia*

## LECTURE II

“*Symptoms* —In considering the symptoms of endocarditis, it is important to bear in mind the manifold conditions under which the disease may develop. A limited number of cases may be grouped together as forming a primary substantive disease, but in the great majority the affection is either an associated pathological state, or is of the nature of a secondary malady arising in the course of some other disease ”

This is followed by a discussion of the modes of onset in which again the embolic complications are stressed, and both retinal hemorrhages and hematuria are referred to. Attention is also directed to the ague types of fever, which, in later communications, plays an important role

“The different modes of onset, and the extraordinary diversity of symptoms which may arise, render it very difficult to present a satisfactory clinical picture. The general symptoms are those of a febrile affection of variable intensity, which may be ushered in, like any acute fever, with rigors, pain in the back, vomiting, headache, etc. Arising in the course of some other disease, there may be simply an intensification of the fever, or a change in its features. The pyrexia is constant, but variable in type and intensity, and more likely than any other symptom to lead to misinterpretation. Prostration of strength, delirium, sweating, and other signs of severe constitutional disturbance, are usually present

Cardiac symptoms may be marked from the outset, pain, palpitation, sense of distress, and murmur, in many instances, there has been old valvular disease, but

in a considerable number of cases the heart-symptoms remain in the background, hidden by the general condition, and giving no indication, or they may be so slight, that they are not even detected on special examination

The embolic processes give a special prominence to local symptoms, which may divert attention from the general malady. Thus delirium, coma, or paralysis may arise from implication of the brain or its membranes, pain in the side and local peritonitis from involvement of the spleen, bloody urine and pain in the back from affection of the kidney, loss of vision from retinal hemorrhages, and suppuration in various organs, or gangrene, from the distribution of emboli

So diverse are the features of malignant endocarditis, that a consideration of the symptoms is greatly facilitated by arranging the cases in groups, according as they display special characters. Dr Kirkes, in 1852, called the attention of the profession to the occurrence of a typhoid-like condition in acute endocarditis, and he subsequently pointed out the fact that inflammation of the valves might lead to pyaemia. The investigations of Charcot and Vulpian (*Gazette Médicale de Paris*, 1862), of Virchow (*Gesammelte Abhandlungen*), of Jaccoud (*Nouveau Dictionnaire de Médecine*, etc., art. Endocarditis), and others, gradually led to the recognition of these two great types of the disease. Of late, still further separation has been made of the cases with features closely resembling ague or intermittent, and also of cases in which the cardiac symptoms are most prominent, and I shall call attention to certain cases in which the symptoms are those of an acute affection of the cerebro-spinal system. "

A little later in the lecture considerable space is devoted to an account of this ague type of the disease which Osler notes may be prolonged for several months

"But by far the most remarkable cases of the pyaemic group are those which present a marked intermittent type of pyrexia, simulating a quotidian or tertian ague. They may occur without any signs or indications of heart-disease, or the symptoms may develop in individuals the subjects of chronic valvulitis. The cases are not nearly so frequent as those of the typhoid type, but they have been specially studied by Drs Wilks, Bristowe, and Coupland in this country, Lance-reaux in France, Leyden and others in Germany. The paroxysms may have the absolutely typical features of intermittent, the chills, hot stage, and sweating succeeding each other with regularity, and in the intervals there may be an entire absence of the fever. The quotidian type is the most common, the tertian has occasionally been described, and in rare instances two paroxysms have recurred within the twenty-four hours. The cases may be much prolonged, even for three or four months. One of the first references I find to cases of this kind is in a footnote to one of Dr Ormerod's *Gulstonian Lectures* (*Medical Gazette*, 1851), in which a case of Dr Bond of Cambridge is narrated—an instance of chronic valvular disease, with intermittent fever and diarrhoea, two paroxysms occurring in the day. The case lasted four months. In a remarkable case (Dr Ray) described by Dr



Wilks (BRITISH MEDICAL JOURNAL, 1868), during a six or seven weeks' illness, rigors recurred with such regularity that a tertian ague was suspected for a time, although the patient was known to be the subject of heart-disease. In some instances, the existence of ague previously has rendered the condition much more puzzling. In several of Lancereaux' cases (*Gazette de Médecine*, 1862, *Archives Générales*, 1873), the patients had had intermittent fever a short time before, so also with one of Leyden's cases (*Zeitschrift für Klin Med*, Bd iv, Berlin). But the most extraordinary case of the kind is recorded by Dr Bristowe (*British Medical Journal*, 1881). A patient had ague in October, chills once or twice a day, she was ill for six weeks, and, after an interval of two or three weeks, they recurred in the second week of December, and continued until December 23rd. She was well for a few days, and then the attacks recurred after sleeping in a cold bed, and persisted until her admission to hospital on February 12th. For the four weeks previous to entrance, the attacks came every twelve hours regularly. A murmur was noticed, but the history of ague was so clear, and the attacks so characteristic, that a suspicion of malignant endocarditis was at first not entertained. It was only after the failure of quinine and a variation in the character of the paroxysms, that a diagnosis was reached. In Dr Coupland's cases (*Med Times and Gazette*, 1822, vol i), the intermittent pyrexia was also well marked. In none of our Montreal cases was the aguish type very pronounced, though in one or two cases there were regularly recurring paroxysms of chills, fever, and sweating, but the conditions under which the attacks developed rendered the clinical features more like ordinary pyæmia. The majority of these cases appear to arise independently of other affections, and occur among what I have referred to as the primary class of cases, though, as already mentioned, some develop in chronic valvular disease, and others appear associated in some way with ague."

The following cases illustrate the chief features of this form

"Ann O, aged 46, large well nourished woman, was admitted under Dr Wilkins, June 5th, 1881. She had been a healthy woman. Dr Blackader saw her on the 2nd, when she complained of severe pains in the back, loins, and hips, which were relieved by poultices. Pulse rapid, tongue furred, no diarrhoea. She was supposed to be suffering from typhoid fever. No reliable history, family or personal, could be obtained, but she had been out of sorts for four or five days previous to the onset of the attack. On admission, temperature 104°, pulse 110, respiration 32, no eruption, lungs normal, no heart-murmur, no albumen in urine. On the 6th, she passed a restless night. Temperature, 104°, pulse 120, dicrotic, abdomen distended, two stools. She passed 18 ozs of urine, slightly bloody, which might have been from the menses, which began to day. On 7th, morning-temperature 103.2°, pulse weak, 120, respiration 54, shallow, loud sonorous râles over chest, bowels and bladder emptied involuntarily, stools frequent, high coloured, patient could not be roused. The legs and general surface seemed tender, which caused her to cry out when moved. Urine drawn off by catheter contained much blood,

50 per cent by volume of albumen, and many granular casts Pupils unequal, head drawn to the right Some rigidity of muscles of arms, most marked on the left, increasing coma, and death at 3 30 P M of the 7th, the sixth day of her serious illness At necropsy, no hypertrophy of heart, mitral valves a trifle thick, with small superficial losses of substance on both curtains Aortic valves normal, infarcts in spleen Numerous small haemorrhagic emboli in kidneys and throughout the intestines Six or eight suppurating infarcts in brain, chiefly near longitudinal fissure and on median surfaces The case is a good example of the primary malignant endocarditis occurring in a healthy individual, and running a rapid course, with symptoms of typhoid character The diarrhoea was not profuse, though the intestinal lesions were well marked "

There then follow descriptions of acute cases occurring during the course of pneumonia or in pyaemia, but in the next group termed "Cardiac Group" Osler refers to cases that remind one very forcibly of instances of subacute or even chronic bacterial endocarditis

"*Cardiac Group*—Under this heading may be arranged, as suggested by Dr Bramwell (*Diseases of the Heart*), those cases in which patients, the subjects of chronic valve-disease, are attacked with febrile symptoms and evidences of a recent endocarditis engrafted upon the old process I have already remarked on the great frequency with which ulcerative changes are found in connection with sclerotic endocarditis Many of such cases present features of the pyaemic, typhoid, or cerebral types, and may be of the most acute character, but, in others, the process appears much less intense, and the cause more chronic In a considerable series of cases, the history is somewhat as follows The patient has, perhaps, aortic valve-disease, and is under treatment for failing compensation, when he begins to have slight irregular fever, an evening exacerbation of two or three degrees, some increase in cardiac pain, and a sense of restlessness and distress Embolic phenomena may develop, a sudden hemiplegia, pain in the region of the spleen, and signs of enlargement of the organ, or there is pain in the back, with bloody urine In other instances, peripheral embolism may take place, with gangrene of the foot or hand There may be hebetude or a low delirium Instances such as these are extremely common, and while, in some, the process may be very intense, in others the term malignant seems not at all applicable to them, still, in a large series of cases, all gradations can be seen between the most severe and the milder forms Dr Green (*Lancet*, 1884, vol 1) referred to a case which lasted six months, and to another in which, during eighteen months, there were attacks of irregular fever I have known the febrile symptoms subside for weeks, to recur again with increased severity, and there are cases which render it probable that the process may subside entirely The ulcerative destruction, in these cases, may be most extensive, and I have seen the aortic ring with scarcely a trace of valve-substance left The process in the chronic cases is also mycotic, and it is to be

carefully distinguished from the atheromatous changes In very many instances, there is no history of rheumatic fever or of other constitutional disorder, but the endocarditis appears to attack the sclerotic valves as a primary process, and a very considerable number of the most typical cases are of this kind A good example was the following case, in which the disease attacked perfored and hardened valves, and the clinical symptoms were prolonged for nearly three months

H M , aged 38, was admitted September 8th, under Dr Ross He had a good family and personal history, he had always enjoyed excellent health A month ago he had chilly feelings, fever, and sweating, with vomiting He kept about until ten days before admission, when he took to bed, with pains at the heart, and fever On admission, there was marked aortic incompetency, temperature 100° Fahr , he seemed dull and heavy On 15th, there was iliac tenderness, and some diarrhoea For the next two weeks, he remained in same state, temperature rising at times to 103° Fahr During the first week of October, the prostration increased, and there was slight delirium at night, temperature not higher than 102° Fahr On the 14th, there was an eruption of petechiae From this time, the temperature kept lower—100° to 101° Fahr—the delirium and prostration increased, and death took place on the 23rd Two of the aortic cusps had fused, and there were old sclerotic changes, there were recent soft greyish vegetations, the spleen presented six or eight infarcts, one suppurative

These are the cases of ulcerative endocarditis which present fewest difficulties in diagnosis The existence of the chronic heart-disease excites attention, and even if compensation has previously been perfect, the ulcerative process may be the very cause of disturbing the balance and producing marked symptoms In my experience, the existence of fever is invariable when the ulcerative processes are due to micrococci, whereas most extensive destructive changes may occur in atheromatous disease without any elevation of temperature It may be possible that the granular detritus discharged from atheromatous foci on the valves, or on the aorta, may have irritating properties, yet, in two instances, I have met with most extensive atheromatous ulcers on valves and aorta, from which large quantities of material must have been discharged, and the patients were not febrile Dr Sansom (*Lancet*, 1884, vol i), however, has referred to a case of ulcerative endocarditis in which there was no elevation of temperature throughout "

The so-called "cerebral group" which follows, is composed principally of cases running an acute course with cerebra-spinal meningitis

In the description of the cutaneous lesions definite reference is made to "petechiae "

"The occurrence of a rash has been described by many observers and, in some instances, has led to errors of diagnosis The most common form is the hemorrhagic, in the form of small petechiae, distributed over the trunk, particularly the abdomen, less often in the face and extremities They may be most abundant over the whole

body, and at times are large and present small white centres. When severe nervous symptoms are also present, the resemblance of the cases to cerebro-spinal meningitis, or typhus, may be very close. In one instance, the case was thought to be hæmorrhagic variola (Duget and Hayem, *Comptes rendus de la Soc. de Biologie*, 1865). An erythematous rash has also been observed.

In a case of Dr. Cayley's (*Lancet*, 1844, 1), there was a mottled red rash on the skin. Colson (*Bull. de Soc. d'Anatomie*, 1876) describes a case in which the rash was erythematous, and in spots distinctly papular. "

This lecture ends with a paragraph alluding to the variable course of the disease, and again Osler makes passing reference to the cases lasting two to three months.

"The course of the disease presents many variations, well illustrated by the records I have given, very acute cases may run their course within the week, as in the patient Ann O., already referred to, while in others the duration may be even two or three months. Except in certain cases in which the patients are the subjects of chronic valvulitis, the course is rarely prolonged beyond four or five weeks."

The third and last lecture starts with a paragraph emphasizing the difficulties of diagnosis.

### THE GULSTONIAN LECTURES, ON MALIGNANT ENDOCARDITIS

Delivered at the Royal College of Physicians of London, March, 1885

By WILLIAM OSLER, M.D.

*Professor of Clinical Medicine at the University of Pennsylvania, Philadelphia*

#### LECTURE III

"*Diagnosis*—Few diseases present greater difficulties in the way of diagnosis, difficulties which in many cases are practically insurmountable. It is no disparagement to the many skilled physicians who have put their cases upon record to say that, in fully one-half of them, the diagnosis was made *post mortem*. In spite, too, of able memoirs in the journals, the disease has not been much known, and it is only of late years that the text-books have contained chapters upon it. The protean character of the malady, the latency of the cardiac symptoms, and the close simulation of other disorders, combine to render the detection peculiarly difficult.

In the group of cardiac cases in which the disease attacks a patient the subject of chronic valvulitis, the matter is usually easy enough. The existence of fever of an irregular type, and the occurrence of embolism, generally suffice to make the

case clear It must be remembered that simple warty endocarditis not unfrequently attacks sclerotic valves, and may be accompanied by slight fever Of course, in chronic heart-disease, irregular pyrexia may arise from other causes—local suppuration, cellulitis, etc—which must be excluded ”

Among these difficulties, as Osler points out later, one relates to the resemblance that certain types of this disease bear to malaria

“It seems strange that difficulties should arise in the diagnosis between malaria and malignant endocarditis, but the records of cases plainly show that for weeks or months a condition of intermittent pyrexia may occur, simulating every type of ague The paroxysms in regularity, in order of sequence, and in the accompanying general conditions, may fulfil every condition of a quotidian or tertian intermittent, and the development of cardiac symptoms, with breathing of the pyrexial type, may alone determine the nature of the case ”

In the long discussion devoted to the etiology of infective endocarditis, rheumatism assumes a prominent position But it does so, not as much on account of its being an immediate cause of the acute disease, as by reason of the fact that some of the patients gave a history of having had a previous attack

“In 127 of the cases, the endocarditis was associated with other diseases, some of the most important of which we shall now proceed to consider

*Rheumatism*—Since Bouillaud called special attention to the frequency of cardiac complications in this disease, its importance in the etiology of endocarditis has been universally recognised And, as regards the simple form of endocarditis, the general statements are quite true, but, fortunately, the graver and fatal form is much less common, much less, I think, than is usually supposed In 53 cases, there was a history of rheumatism, past or present I included every case in which there had been the record of an attack, recent or remote In only 24 did the symptoms of severe endocarditis arise during the progress of the acute or sub acute disease In 29 cases, there was simply a history of rheumatism, often years before, and no mention of the occurrence of joint-troubles at the time of the development of the endocarditis Dr Ogle called attention to the fact that ulcerative endocarditis occurred very often in persons in whom no rheumatic history could be traced Of 21 cases which he reported, some of which were probably atheromatous, in only 3 was rheumatism mentioned In only 3 also of the Montreal cases was there any positive history of rheumatism, either before or during the attacks The following case, under the care of Dr Ross, is a good example of the mode of onset

“In a larger number than in any other group, sclerotic valves were found, with the existence of which the past rheumatism could, in many instances, be connected

A primary rheumatic endocarditis was recognised by Latham, also by Graves and Stokes, and it is quite possible that some of the cases which I have grouped as prothopathic represented instances of the kind in which, if life had been prolonged, joint-troubles might have supervened "

The final paragraphs give some conception of the difficulty encountered in solving the etiology of such a complicated situation at a time when the science of bacteriology was in its infancy

"Briefly stated, the theory of acute endocarditis which at present prevails, and the only one to which I shall refer, is, that it is in all its forms, an essentially mycotic process, the local and constitutional effects being produced by the growth on the valves, and the transference to distant parts of microbes, which vary in character with the disease in which it develops This very attractive theory can be adjusted to meet every requirement of the case, though as yet lacking certain of those substantial data necessary for full acceptance, but which, having been furnished of late years in other diseases, we may reasonably hope will in time also be forthcoming for this

In the way of experimental investigation of the properties of the micrococci, not much has been done of a satisfactory nature Heiberg (*Virchow's Archiv*, Band lvi) placed bits of vegetations from a puerperal case beneath the skin and in the peritoneal cavity of a rabbit without effect Eberth (*Ibid*, Band lvii), Birch-Hirschfeld (*Archiv der Heilkunde*, Band xvii), have produced panophthalmos in the rabbit by inoculating the cornea, and I was able to produce well marked mycotic keratitis in the same animals with fresh material from the valves of two cases H Young, of Manchester, inoculated rabbits with pus from an abscess in ulcerative endocarditis, and was able to detect micrococci in the blood

No conclusive culture-experiments have yet been made Grancher (*Journal de Médecine de Paris*, December 20th, 1884) has cultivated a microbe from the blood, taken during life with all necessary precautions, but apparently not in series, and no inoculations of animals were made Cornil (*L'Abeille Médicale*, December 22nd, 1884) has made cultures on gelatine, but apparently no special results have been reached

In the first place, we do not yet know, with sufficient accuracy, the frequency of the occurrence of microbes in simple endocarditis Are they constantly present, or only in forms associated with special diseases? Secondly, we want full information of the various forms of micro-organisms occurring in secondary endocarditis, and of their relation to the microbes assumed to be the cause of the primary disease And, thirdly, we are only at the threshold of inquiries relating to the culture of these organisms, to the macroscopic characters of their growth, and to the possible experimental production of endocarditis "

These lectures remain today a classical contribution to the subject of infectious endocarditis They incorporated all the knowledge that

was then available concerning this subject, and were still further illuminated by Osler's personal observations. He correlated this information and classified it as well as it could be done at a time when bacteriology was in an embryonic state. Though, perhaps, a majority of the cases were instances of acute infectious endocarditis, several could now be interpreted, from the description which Osler gives, as examples of subacute bacterial endocarditis. The preëxisting valvular lesions, the prolonged course with irregular or intermittent fever, the subjective symptoms, the petechial hemorrhages in the skin, the retinal hemorrhages, enlarged spleen, the hematuria and the presence of multiple and non-suppurative infarcts are all characteristic of this condition.

In the year following the Gulstomia Lectures Osler published an important paper which is rarely referred to. It was entitled "The Bicuspid Condition of the Aortic Valves" (5) and appeared in the first volume of the Transactions of the Association of American Physicians. He defines the condition as follows:

### THE BICUSPID CONDITION OF THE AORTIC VALVES

BY WILLIAM OSLER, M D , F R C P , LOND

*Professor of Clinical Medicine in the University of Pennsylvania*

(Trans Assoc Am Physicians 1886 i, 185-192)

*Definition*—A condition of the arterial valves in which two of the cusps are more or less perfectly fused, so that the orifice is guarded by only two segments.

*Frequency of the Occurrence*—It is usually referred to as a common abnormality, and Dilg<sup>1</sup> has tabulated 64 cases in the pulmonary, and 23 in the aortic, valves. A careful study of the anomaly has recently been made by Martinotti and Spornio,<sup>2</sup> and again by Martinotti,<sup>3</sup> who remarks that the list given by Dilg of the condition in the aortic valve might be greatly extended. In over eight hundred autopsies, I have met with it in 18 cases, 17 in the aortic valves, and in 1 case in both aortic and pulmonary valves. A detailed account of the cases is given in the appended table. In 110 cases of valvular disease of all kinds, there were 57 in which the aortic segments were affected, either alone or in conjunction with the mitral and tricuspid valves, so that this condition was present in over thirty per cent. of all the cases of aortic disease, a proportion which I am inclined to regard as exceptional."

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<sup>1</sup> Virchow's Archiv, xci

<sup>2</sup> Atti della R. Accademia di Medicina di Torino, 1884, reprint

<sup>3</sup> Gazzetta delle Cliniche, 1886, reprint

He then goes on to say that the aortic valves in 8 of his 18 cases were the seat of an infectious endocarditis, a matter to which he had referred in an earlier paper. He now elaborates still further this statement

*Clinical Features*—In two of the Cases (2 and 14) the condition was found after death and there was no evidence that the persons had suffered from cardiac symptoms. Cases 5 and 10 died suddenly, and in Case 4 death was also sudden but resulted from the rupture of a cerebral aneurism. Excluding Case 7, a foetus, the remaining twelve cases presented the clinical features of heart disease. In eight there was ulcerative endocarditis, in Cases 7, 9, 13, and 18 of a very severe type. Cases 1, 11, 15, and 16 were examples of gradual heart failure with the usual symptoms of disturbed compensation. Thus in fifteen<sup>1</sup> of the cases the cause of death could be attributed directly or indirectly to the existence of this anomaly. Whether the result of foetal endocarditis or a primary failure in development, the condition thus plays an important part in the history of aortic valve disease. The special proneness of mal-formed structures to disease is well known, and the conjoint segments are rarely, in the adult, free from sclerotic changes, while in nearly half of my cases there was also ulceration. Doubtless, the strain upon the fused curtains is more severe than upon normal cusps, and though in the foetus, and even in the adult, the tissue of the valve may have the natural thinness and mobility, yet, as a rule, there is induration and thickening. I have elsewhere<sup>2</sup> called attention to the frequency with which ulcerative endocarditis attacks sclerotic valves. Indeed, it is exceptional for normal segments to be affected. The recent investigations in experimental endocarditis by Orth<sup>3</sup> (which are confirmed by the beautiful demonstration of Prudden at this meeting) would indicate that the micrococci cannot lodge on the normal valve, but the slightest abrasion suffices to permit of their entrance. Although Ribbert<sup>4</sup> has been able to induce endocarditis by injecting cultures of the microorganisms without any previous lesion of the valves, particularly if the material was associated with rougher particles, these recent experiments support the experience of the post-mortem room that a damaged valve is the most likely to become the seat of ulcerative changes.”

There follows a short discussion of the origin of this anomaly

*Origin*—Whether the condition is the result of a foetal endocarditis or is an anomaly of development cannot be finally settled until we have fuller knowledge

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<sup>1</sup> In case 4, a lad of twenty, in which rupture of a cerebral aneurism was the immediate cause of death, there was evidence of the connection of the aneurism with a previous embolic process, which might reasonably be associated with the valve lesion

<sup>2</sup> British Medical Journal. Gulstonian Lectures, 1885, vol. 1

<sup>3</sup> Tageblatt der 58 Versammlung Deutscher Naturforscher zu Strassburg, 1885

<sup>4</sup> Deutsche med. Wochenschrift, 1885, No. 42



of the details of formation of the semilunar valves. The advocates of the inflammatory view urge that indications of the original separation invariably exist and that the valves as constantly present evidences of endocarditis. To this view Virchow has given the weight of his authority and has recently<sup>1</sup> stated that an examination of the question has convinced him that a majority of the cases show signs of a "secondary fusion of two cusps." This certainly may be so in some cases, but the following considerations lead, I think, to the conclusion that in many there is a faulty arrangement at the time of the development of the segments."

Osler at the very end of the paper, suggests that the anomalous condition of the aortic valves may be due to an error in their development.

"Fourth. If it turns out to be correct, as my cases indicate, that the affected valves are usually those behind which the coronary arteries are given off, this would point to some error associated especially with the development of these cusps."

He finally tabulates the pathological findings in his 18 cases and Nos 9, 11, 13 and 18 are quoted from this table on account of their particular interest.

NO	SEX AND AGE	CAUSE OF DEATH	STATE OF HEART	AORTIC VALVES	OTHER ORGANS	REMARKS
9	M 45 (?)	Ulcerative endocarditis	Much hypertrophied weight 600 grms	Coronary segments united measure 4.5 cm. Sinuses of equal size. Raphe small. Shallow groove on ventricular surface at attachment of valve. Moderate sclerosis. Inter coronary segment covered with vegetation and presents a perforation 2 by 1 cm.	Infarcts in spleen	In hospital four days. Malignant endocarditis.
11	M 35 (?)	Ulcerative endocarditis	Much hypertrophy	Coronary segments united and sclerotic. Behind them a recent aneurism which projects into, and communicates with, left auricle. Vegetations on ventricular face. Inter coronary segment much thickened, and presents recent vegetations.	Great enlargement of spleen weight 500 grms	Patient ill several months.
13	M 38	Ulcerative endocarditis	Hypertrophy and dilatation	Coronary segments fused. Edge much thickened. Sinuses large and the raphe scarcely visible. Inter coronary segment presents many recent vegetations and is a little thickened.	Extensive recent disease of mitral valve infarcts	Marked typhoid symptoms.
18	M 26	Ulcerative endocarditis apoplexy	Hypertrophy of left ventricle	Fusion of coronary segments. Sclerotic changes. Vegetations on ventricular face. Perforation of valve. Vegetations on inter coronary cusp.	Infarcts	Case simulated typhoid fever.

<sup>1</sup> Virchow's Archiv, Bd 103

It seems probable that if not all, at least some, of these cases were examples of congenital bicuspid aortic valves, and if so this paper forms one of the earliest observations upon the occurrence of acute or subacute endocarditis in this condition. It was 37 years later that Lewis and Grant (6) published their well known article on the subject and 39 years before Maud Abbott (7) wrote extensively on inflammatory Processes in Cardio-vascular defects.

During the years immediately following the appearance of these papers, from which quotations have been so freely taken, Osler discussed the question of infectious endocarditis, according to Maud Abbott's "Bibliography of Osler," at medical meetings and in several unsigned editorials. For instance, he presented, some time between 1887 and 1889, two cases of ulcerative endocarditis (8) before the Path. Society of Philadelphia, one of which was supposed during life to be an instance of advanced "phthisis" in a young woman of 30 with heart disease.

There were, however, many other important matters to engage his attention. In 1889 he left Philadelphia to become Professor of Medicine at the Johns Hopkins Medical School and between 1890 and 1892 he was immersed in the absorbing work of writing his text book.

The first edition of the text book appeared in 1892. In it there is quite a long section on *Acute Endocarditis*. This is constructed very largely on the basis of his Gulstonian Lectures and his previous papers on the subject. There is no need to discuss this excellent and concise account of acute endocarditis as far as it was then known in detail, but it is noteworthy that at that time, he did not mention any specific bacteria as the causative agents of infectious endocarditis. It is important, on the other hand, to draw attention to what appears to be a growing realization that there was one type of case in which the course of the disease was surprisingly long. In the paragraph dealing with the "septic type" there occurs the following statement:

"In a most remarkable sub-group of this type the disease may simulate a quotidian or tertian ague. The symptoms may develop in persons with chronic heart-disease without any external lesions. These cases may be much prolonged—for three or four months, or even longer, as in a case of Bristowe's. The existence in some of these instances of a previous genuine malaria has been a very puzzling circumstance."

And again in referring to the progress of acute endocarditis he states

"The course of the disease is varied, depending largely upon the nature of the primary trouble. Except in the disease engrafted upon chronic valvulitis the course is rarely extended beyond five or six weeks. As already mentioned, there are instances in which the disease is prolonged for months."

In spite of his many exacting and varied duties, Osler evidently continued his studies of infectious endocarditis for in 1893, the very year that the Medical School opened, he published a paper in which he drew particular attention to the protracted course of the disease in two patients. Osler's description of these two cases, combined with his comments, leads one to believe that he was beginning to discern that there was or might be a particular type of infectious endocarditis, which, under the name of subacute bacterial endocarditis, is very familiar to us today.

The article starts, as usual, with references to Wilks, Bristowe and others, with a summary of the clinical features of the two cases.

#### THE CHRONIC INTERMITTENT FEVER OF ENDOCARDITIS (9)

BY WILLIAM OSLER, M D , F R C P LOND

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(Practitioner, London 1893, 1 181-190)

"The type of endocarditis characterised by a protracted course and an irregular intermittent fever has been specially studied by Wilks, Bristowe, Coupland, and Lancereaux. In my Gulstonian Lectures (1885) its characters are thus described: The paroxysms may have the features of ague, the chill, hot stage, and sweating succeeding each other with regularity, and in the intervals there may be an entire absence of the fever. The quotidian type is the most common, the tertian has occasionally been described, and in rare instances two paroxysms have recurred within the twenty-four hours. The disease may be much prolonged, even to three or four months.

One of the first references I find to cases of this kind is in a footnote to one of Dr Ormerod's Gulstonian Lectures,<sup>1</sup> in which a case of Dr Bond, of Cambridge, is narrated—an instance of chronic valvular disease, with intermittent fever and diarrhoea, two paroxysms occurring in the day. The case lasted four months. In a remarkable case described by Dr Wilks,<sup>2</sup> during a six or seven weeks' illness,

<sup>1</sup> *Medical Gazette*, 1851

<sup>2</sup> *British Medical Journal*, 1868

rigors recurred with such regularity that a tertian ague was suspected for a time, although the patient was known to be the subject of heart disease. In some instances, the existence of ague previously has rendered the condition much more puzzling. In several of Lancereaux's cases<sup>3</sup> the patients had had intermittent fever a short time before, so also with one of Leyden's cases.<sup>4</sup> But the most extraordinary case of the kind is recorded by Dr Bristowe.<sup>5</sup> A patient had ague in October, with chills once or twice a day, in an illness of six weeks. After an interval of two or three weeks they recurred in the second week in December, and continued until December 23. She was well for a few days, and then the attacks recurred after sleeping in a cold bed, and persisted until her admission to hospital on February 12. For the four weeks previous to entrance, the attacks came every twelve hours regularly. A murmur was noticed, but the history of ague was so clear, and the attacks so characteristic, that a suspicion of malignant endocarditis was at first not entertained. It was only after the failure of quinine, and a variation in the character of the paroxysms, that a diagnosis was reached. In this case, the most protracted with which I am acquainted, the condition persisted for more than five months, and Dr Bristowe has informed me that he regarded the case as one of ulcerative endocarditis from the outset.

I have recently had under observation a remarkable case in which the symptoms persisted for nearly ten months, and through the kindness of Dr Mullin of Hamilton, Ontario, I am able to give the notes of a second case in which the disease continued for eleven months. The clinical features of these two cases may thus be summarised.

(1) Daily intermittent pyrexia for many months, the temperature rising to 102° 5 and 104°, occasionally preceded by a distinct rigor, more commonly by feelings of slight chilliness. Following the pyrexia there was more or less sweating.

(2) Progressive failure of strength, with varying intervals of improvement.

(3) Physical signs of cardiac disease—in the cases here reported an apex systolic murmur, with hypertrophy of the left heart.

(4) Development towards the close of the embolic symptoms more usually associated with ulcerative endocarditis, and cutaneous ecchymoses."

The first patient was a man of 43. Early in December, 1891, he suddenly fell ill with a chill and fever followed by malaise, fatigue, cough, pains in different parts of his body and loss of weight. The patient was under observation from March 15 to May 10. During this period he had irregular fever, signs of a lesion of the mitral valve, and pain and tenderness in the splenic region, though the spleen was

<sup>3</sup> *Gazette de Médecine*, 1862, *Archives Générales*, 1873.

<sup>4</sup> *Zeitschrift f klin Med*, vol iv. Berlin.

<sup>5</sup> *British Medical Journal*, 1881.

not palpable When he was discharged from the Johns Hopkins Hospital the following final note was made

“ Repeated examinations showed no apparent change in the cardiac condition The intense systolic murmur at the apex, obliterating the first sound, persisted No increase could be determined in the area of cardiac dullness The sounds in the aortic region remained clear The patient left the hospital on May 10, and the history chart was headed “chronic vegetative endocarditis ”

The subsequent history is interesting on account of the continuous fever and emaciation, the appearance of petechiae and the late urinary findings

“For the subsequent history I am indebted to Dr Block, who has sent the careful temperature chart kept by the nurse up to the time of the patient's death From this it may be gathered that the temperature range throughout May and June was from 97° to 103° In July the average was decidedly lower, and towards the end of the month he had several days when the temperature was almost normal Early in July petechiae appeared, and several groups of these were noticed On August 19 the temperature became normal, and remained so until the 24th, but the pulse was weak and he had free sweats During the first week in September the temperature was usually sub-normal, and only reached 98° in the evening The morning temperature was frequently 95° There were profuse perspirations From the 9th until his death on the 14th the temperature only once registered 98°, and for four days was continuously below 96° He failed progressively, became extremely emaciated, had diarrhoea, and there were blood-corpuscles and blood casts in the urine The pulse was feeble, irregular, and intermittent There were no brain symptoms, and he remained conscious until the last ”

At autopsy there were enormous vegetations on the mitral valve, slight enlargement of the spleen with anaemic and softened infarcts, enlarged kidneys showing infarcts, and petechial hemorrhages over the peritoneum No bacteriological examinations or cultures were made

The description of the disease in this patient conforms very well to a case of subacute bacterial endocarditis, while in the second patient the clinical picture is even more characteristic, and therefore sections of the history and examination are quoted in full

“ The patient has generally enjoyed good health, but at twelve years of age she had an attack of rheumatism, apparently not severe, as she was in her room only one week, and not in bed all of the time About four years before the onset of her last illness she had pain and slight swelling in one knee, was not confined to

bed, but wore a splint for a week. She has always been pale, and when at boarding school her teacher often suggested that iron would be of use. She, however, did not feel ill, and scarcely ever thought that she required medical treatment. At times, however, upon some sudden exertion she felt a stabbing pain in the region of the heart which never lasted long. The menses were always regular until the early part of the illness. In February 1888, she caught cold when tobogganing, and had pain in the back part of the chest, but did not require to go to bed. In March she visited some friends at Niagara Falls, where she remained until July. Here her friends noticed that she looked miserable for some time before she spoke of being ill. The menses failed to appear, and she thought this was the reason why she did not feel so well as usual. She sometimes had attacks of faintness, which soon passed away on taking a stimulant. She became weaker, and had fever followed by night sweats, the fever came on in the afternoon. A physician was consulted, who said the heart was affected and that she required prolonged medical treatment and rest. She continued, however, to go about, and frequently took long walks, though on exertion she complained of being short of breath. She had fever and sweating at night, and was often so restless that she was obliged to leave her bed and recline on the sofa. "

" In the first week of July she came home, and was placed under my care. In the forenoon the temperature appeared normal, but every afternoon it rose to 102° or 103°. For a time she was thought to have typhoid fever, but no distinctive symptoms appeared. "

" No local symptoms arose to account for the fever, pain was not complained of to any great extent, sometimes, for a few hours or half a day, there would be aching and pain in the hands and different joints, but these were always transient and at no time after she came home was there marked tenderness or swelling in any of the joints. When she reached home there was some swelling of the ankles and knees, but this soon passed away as she remained in bed. Not making any exertion she did not suffer from dyspnoea. There was a loud systolic murmur at the apex, and from the first the signs of hypertrophy showed that mitral disease had existed for some time. Before she came home it was noticed at the outset of the illness that small spots appeared on the hands and feet, also on arms and legs and face, that looked like "hives." These continued to appear, they were erythematous, some as small as a pea, others as large as a five-cent piece, with a white point in the centre. They often passed away in a few hours, and never lasted longer than the evening of the day on which they appeared. They were not numerous, sometimes they would appear near the tips of the fingers which for a short time became swollen. These spots were seen more or less throughout the illness, though more of them were noticed in the early part. "

" A careful temperature record was kept in this case from July 17, 1888, until July 7, 1889. The type of fever was in each month remarkably uniform,

the morning record always at or below the normal point, and the evening record reaching 102° 5, 103°, and sometimes 104° At intervals for a week or two the evening temperature did not fall below 100° "

"The *autopsy* showed moderate enlargement of the heart, due chiefly to hypertrophy and dilatation of the left ventricle The aortic valves were normal, the mitral orifice readily admitted two fingers, the valve segments were thickened and presented numerous large vegetations, chiefly on the auricular surfaces, and extending from the base of the posterior segment to the wall of the left auricle The chordae tendineae were a little shortened and thickened, and many of them encrusted with the vegetations The spleen and kidneys contained numerous infarcts in all states of change "

"The diagnosis of these protracted cases is often very difficult, and not unnaturally they are mistaken at the outset for malarial fever, particularly when daily chills occur

One very important observation in this second case was the presence of a generalized eruption which also occurred at times near the tips of the fingers Both of these cases are referred to by Osler in a later paper as instances of "Chronic Infectious Endocarditis " He pointed out then that the eruption observed in the second case was the first instance of this particular sign, now known as "Osler's nodes," that had come to his attention It was not, however, until 15 years had elapsed that this later communication appeared

In the meantime he had been offered and had accepted the Regius Professorship of Medicine at Oxford He left Baltimore to make his home in England in the spring of 1905

An event took place the following year which has had great influence upon the development of clinical medicine, not only in England but in the world at large It can best be described by quoting a page from Cushing's *Life of Sir William Osler* (3)—Vol II, page 49

"There was a movement on foot, about the time of Osler's transfer to England, fostered chiefly by Drs Wilmot Herringham, A E Garrod (now Osler's successor), Wilham Hale-White, H D Rolleston, J Rose Bradford and Robert Hutchison, to start a new medical journal of a type rather different to any then being published in England, and, recognizing how great would be the value of his support, Osler was approached on the subject To judge from contemporary letters, he had another project in mind and saw the chance of fusing his scheme with this other one Accordingly, at the preliminary meeting held on May 23rd at Herringham's house, in the course of the discussion he casually remarked "Why not form a National Association of Physicians first, and let the journal come to be its official

organ?"—adding that the Oxford Press might be prevailed upon to undertake the publication, though it was somewhat out of their line. The suggestion was warmly welcomed, and as an outcome of this informal gathering the Association of Physicians of Great Britain and Ireland, with the Quarterly Journal of Medicine as its official mouthpiece, came into being, and the Oxford University Press made its first venture into the field of medical publications. Though Osler never held an official position in the association he served for the following twelve years, until the time of his death, as one of the editors of the journal, and is said to have been "indefatigable in encouraging its growth, shaping its policies and smoothing out its difficulties."

The first meeting of the Association of Physicians of Great Britain and Ireland was held in London in the rooms of the Royal Medical and Chirurgical Society on May 23 and 24, 1907. At this meeting Osler presented a paper on "Multiple Hereditary Telangiectasis," which appeared subsequently in the first number of the "Quarterly Journal of Medicine," of which Osler had been appointed editor in chief. The second meeting of the Association opened in Edinburgh in June 1908 and it was at this meeting that Osler presented his work on "Chronic Infectious Endocarditis."

At this time Osler was familiar with the work of Harbitz and of Lehnhardt and though he refers to a case in which Horder had used vaccines, he does not mention the investigations of Schottmüller (10) on the differentiation of haemolytic streptococci from streptococcus viridans.

Osler's contribution to the subject was noteworthy. The paper is frequently quoted on account of the fact that, in it he described and emphasized one form of eruption which occurs in subacute bacterial endocarditis, now generally designated "Osler's nodes." But over and above this observation the communication has distinction, for he gives a clear and concise account of a condition which though recognized before this time even by Osler himself, had been ill defined. In this article a disease now very familiar as "subacute bacterial endocarditis" is presented as a definite clinical and pathological entity even though its origin cannot always be attributed to exactly the same variety of bacteria.

For these reasons several excerpts from this paper are quoted in full. It starts as usual with a definition, and with references to similar cases that had previously been reported. In this connection he makes the



statement that when he gave his Gulstonian Lectures he had not seen a case of this type, but one wonders in retrospect whether some of the cases of infectious endocarditis to which he refers in these lectures were not actually rather rapidly progressing instances of subacute bacterial endocarditis. This interpretation is certainly applicable to some of the cases described in his later communications, for it seems highly probable that cases 11, 13, and 18 in his paper on "The Bicuspid Condition of the Aortic Valves" (5) which appeared in 1886 were of this nature, and that the young woman whose illness he described at a meeting of the Pathological Society in Philadelphia about the year 1887 and printed in 1891 (8) might well have had subacute bacterial endocarditis. The following quotations have been selected as being particularly important.

### CHRONIC INFECTIOUS ENDOCARDITIS

BY WILLIAM OSLER<sup>1</sup> (11)

(Quarterly J M Jan 1909, 2 219)

"An endocarditis with fever as its only symptom may be prolonged for weeks or months under many different circumstances. That occasional instances were characterized by a very protracted course was noted by Wilks, Bristowe, Coupland, and Lanceraux. In my Gulstonian Lectures, 1885, I stated that this type had the following characteristics: the fever was irregular and intermittent, resembling ague, the cold, hot, and sweating stages might succeed each other with great regularity, in the intervals fever might be absent, two or three paroxysms could occur in the course of a day. In many of the instances the disease was prolonged to three or four months, and I give the notes of a case of Bristowe's in which the condition persisted for five months. The recurring chills usually led to the diagnosis of malaria and also gave rise to the opinion widely held, particularly by French writers, that ulcerative endocarditis could be caused by this disease. The cases to which I wish to call attention in this communication are of this chronic character, not marked specially by chills, but by a protracted fever, often not very high but from four to twelve months' duration. At the time of the delivery of the Gulstonian Lectures I had not seen a case of this type. In the past twenty years I have seen ten cases of this form, two of which I have already reported (*Practitioner*, 1893). I have put them together in tabular form to indicate their main features.

Following these opening paragraphs Osler proceeds to enumerate the types of bacteria which had been found in association with infec-

<sup>1</sup> Read at the Association of Physicians of Great Britain and Ireland, Edinburgh, June 12, 1908."

tious endocarditis and mentions specifically "The pneumococcic, the gonococcic and the streptococcic forms" After referring to the frequency with which patients suffering from the "chronic" type of disease give a history of old rheumatic lesions of the heart valves he describes the course of the fever which is such a characteristic feature of the disease

" Once established the fever becomes the dominant, and for many months may be the only, symptom This is the most striking peculiarity of the cases Week after week, month after month, the daily rise of one and a half or two degrees may be the only indication there is of an existing mischief In Case 1, in which the fever lasted for thirteen months, the patient's sister, a trained nurse, had decorated the room with yards of the temperature charts, fever with an occasional sweat were the only symptoms The appetite remained good and she lost very little weight There were no embolic features and from month to month there were few, if any, changes in the cardiac condition "

The clinical signs referable to the cardiac lesions were matters of some significance as the following paragraph illustrates

" One of the most striking circumstances is the very slight change in the character of the heart murmur in spite of the fact of most extensive vegetations and alterations in the valves Thus in the case of Dr R T, with the condition of whose heart I had been familiar for fourteen years, the comparison between my first examination in 1889 and that in 1893 showed very little change beyond the slightly greater dislocation outwards of the apex beat In several of the cases the absence of any change in the character of the organ were urged strongly against the existence of endocarditis It is rather remarkable, considering the anatomical changes, that so little alteration may occur in the physical signs "

After a note concerning the embolic complications he goes on to describe the celebrated "Osler nodes "

" One of the most interesting features of the disease and one to which very little attention has been paid is the occurrence of ephemeral spots of a painful nodular erythema, chiefly in the skin of the hands and feet, the "*nodosités cutanées éphémères*" of the French My attention was first called to these in the patient of Dr Mullen of Hamilton, whose description is admirable "The spots came out at intervals as small swollen areas, some the size of a pea, others a centimetre and a half in diameter, raised, red, with a whitish point in the centre I have known them to pass away in a few hours, but more commonly they last for a day, or even longer The commonest situation is near the tip of the finger, which may be slightly swollen" Spots of this character occurred in seven of the cases and in

three at least they were of importance in determining the diagnosis. Thus in the case of Dr. Carroll, the well-known American Army Surgeon, the collaborator with Dr. Reid in the brilliant work upon yellow fever, the presence of these spots appeared to me to clinch the diagnosis. They are not beneath but in the skin and they are not unlike an ordinary wheal of urticaria. In one case they were present in the skin of the flank. I have never seen them haemorrhagic, but always erythematous, sometimes of a very vivid pink hue, with a slightly opaque centre."

This is followed by a discussion concerning the difficulties of diagnosis with a statement regarding the most helpful signs and symptoms that may serve as guides

"By far the most suggestive features are (1) a knowledge of the existence of an old valve lesion. This was present in every one of my series. (2) The occurrence of embolic features, sudden swelling of the spleen, with friction in the left flank, sudden attack of haematuria, embolism of the retinal arteries, hemiplegia or the blocking of a vessel in one of the limbs. (3) The onset of special skin symptoms, purpura, and more particularly the painful erythematous nodules to which I have referred. Present in seven of the ten cases, these are of definite diagnostic import. They are in all probability caused by minute emboli. (4) The progressive cardiac changes, the gradual increase in the dilatation of the heart, the marked change in the character of a mitral murmur, the onset of a loud rasping tricuspid murmur, or the development under observation of an aortic diastolic bruit. With carefully made blood-cultures one should now be able to determine the presence of the septicaemia. This was easily done in three of my more recent cases. The blood-cultures and the presence of the painful erythematous nodules and the occurrence of embolism furnish the most important aids."

There follows a short paragraph on the pathological lesions, and then an account of the results of bacteriological examinations of the blood in these patients

"The organisms responsible for this condition have been carefully studied. In my series cultures were made in six cases. In three they were negative. In two streptococci were present, in one a staphylococcus. While, as a rule, this condition is much more commonly caused by the streptococcus other organisms may be present. Thus Fraenkel has reported one instance of a pneumococcus endocarditis persisting for nearly six months (*Deutsche med. Woch.*, 1900). Of sixteen cases of this chronic form, the clinical course of which extended from four to eight months, Harbitz (*Deutsche med. Woch.*, 1899) found pneumococci in four, streptococci in nine, and in eight other micro-organisms. Lenhartz (*Deutsche med. Woch.*, 1901), who has reported sixteen cases with a duration of from three to seven months, found staphylococci and streptococci the common organisms, the

pneumococcus once and the gonococcus once. In the majority of cases it seems to be a mild streptococcus infection, possibly by a special form. Possibly in some instances there may be a special resistance on the part of the host, but these are points which must be settled by future investigations. These are cases in which the possibility of successful vaccine treatment should be considered. It was tried in two cases of my series, but in both rather late, and in neither did it seem to have special influence. Horder has treated a case of this chronic type with a vaccine prepared from the patient's organism, but without success. The results in the acute forms are discussed by him in the Practitioner, May, 1908. Abstracts of the cases are here given."

The article ends with a detailed account of each one of the 10 cases, two of which had been previously reported in 1893. A table showing the essential data in each case is appended and this appears below.

This paper by Osler appeared in the January 1909 number of The Quarterly Journal of Medicine, and in the April number of the same year, Horder published his article entitled, "Infectious Endocarditis" (12). In this article Horder states that he was stimulated to prepare the material which he was collecting for publication upon hearing Osler's presentation at the second meeting of the Association of Physicians of Great Britain and Ireland.

The analysis which Horder made of the 150 cases, with the numerous, excellent illustrations of pathological lesions was an important contribution to the subject. He noted the fairly frequent association of infective endocarditis with congenital cardiac lesions, quoting illustrative cases, drew attention to mycotic aneurysms, and tabulated the incidence of involvement of the different heart valves. With Andrewes he had previously reported the bacteriological findings in blood cultures from a few cases, but in this paper he lists the results obtained between 1905 and 1908 in 32 cases. In 19 a streptococcus was obtained, in 5 B influenza, in 3 the pneumococcus, in 1 a gonococcus and in 1, staphylococcus albus. He emphasizes the frequency with which streptococci are responsible for the endocarditis, and states that, in 100 cases examined at autopsy at St Bartholomew's Hospital between 1901 and 1908, this organism was cultured in 62 instances. He also points out that the commonest forms of streptococci recovered in culture were not the *S. pyogenes* but less virulent varieties termed "*salivarius*," "*anginosus*" or "*faecalis*", and that these organisms might be present in enormous numbers in the circulating blood.

*Summary of Ten Cases of Chronic Infectious Endocarditis*

NO	NAME	AGE	DATE	FORMER RHEU- MATIC FEVER	OLD VALVE LESION	EARLY SYMPTOMS	TYPE OF FEVER	SKIN SYMPTOMS	EMBOLISM	HEART LESIONS	DURA- TION <i>months</i>
1	J M	28	July, 1888	Yes	Mitral	Fever	Remittent and intermittent	Painful nod- ular ery- thema	None	Mitral endocar- ditis	13
2	T B	43	March, 1892	No	Mitral	Chills and fever	Remittent	Purpura	None	Mitral endocar- ditis	10
3	Florence D	21	March, 1899	Yes	Mitral	Chills and fever	Remittent	Painful nod- ular ery- thema	Brain	No p m	7
4	Mary B	19	June, 1890	Yes	Mitral	Chills and fever	Remittent	Painful nod ular ery- thema	Brain	No p m	5
5	R B	53	May, 1902	No	Aortic	Chills and fever	Remittent with chills	—	None	No p m	4
6	Dr B T	33	Sept, 1902	No	Aortic	Arthritis, chills, fever	Intermittent and remit- tent	Painful nod- ular ery- thema	None	No p m	8
7	Dr R T	53	Feb, 1903	Yes	Mitral	Fever and sweats	Remittent	Painful nod- ular ery- thema	Retina, spleen, kidney	Mitral, aortic and tricuspid endocarditis	8
8	R W	36	Nov, 1906	Yes	Mitral	Chills and anaemia	Remittent	Purpura	None	No p m	6
9	Dr C	52	May, 1907	No	Mitral	Fever	Remittent	Painful nod- ular ery- thema	Brain	No p m	7
10	Alice A	20	Jan, 1908	Yes	Mitral	Fever	Remittent	Painful nod ular ery- thema	None	No p m	7

He then proceeds to divide the cases of infective endocarditis into 5 types (1) Latent infective endocarditis, (2) Fulminating infective endocarditis, (3) Acute infective endocarditis and (4) Subacute infective endocarditis, this, the fourth being the commonest and occurring in 88 of the 150 cases. Finally No 5, chronic infective endocarditis accounted for 18 of the 150 cases. Thus over 70% of the cases belonged to the subacute and chronic groups.

He then discusses the symptoms and signs of the disease, and points out in particular the occurrence of embolism, the frequency of splenic enlargement, the presence of hemorrhagic renal lesions producing the "flea bitten" kidney, the varieties of skin eruption encountered with the diagnostic significance of petechiae, which he found early about the neck and upper chest. He also alludes to the nodes described by Osler.

He makes reference to the retinal lesions, and notes that they may be overlooked, for, like petechiae in the skin, they may be transient.

Some space is devoted to the types of fever encountered, and to the other symptoms which are now familiar.

In the section devoted to prophylaxis and treatment, he suggests that "Oral Sepsis" should receive careful attention, for as he states "Reference has been made to the undoubted fact that the source of the infecting agent in most cases is the mouth or intestine."

In relation to the treatment of this disease Horder used a long list of drugs without effect, anti-streptococcus serum proved useless, and 12 cases treated by vaccines, all died. He concludes that the disease is almost uniformly fatal. Some years later, Horder elaborated this entire subject in his Lumlian Lectures (13).

Though individual instances of subacute or chronic cases of bacterial endocarditis had been reported before the work of Osler, it is fair to say that his contributions together with the paper by Horder established without question the existence of a particular group of infective endocarditides, which since then have been the subject of the most extensive investigations.

The last published communication that Osler made upon infective endocarditis appears to be the report of a clinic conducted at the Radcliffe Infirmary on October 24, 1911. This paper seems rarely to be quoted. It is as follows:

# CHRONIC INFECTIOUS ENDOCARDITIS, WITH AN EARLY HISTORY LIKE SPLENIC ANEMIA (14)

BY SIR WILLIAM OSLER, BART, M D , OF OXFORD

*Regius Professor of Medicine, Oxford University*

"Clinical Remarks at the Radcliffe Infirmary" October 24, 1911

(Interstate Med J 1912, 19, 103 St Louis, Mo)

The subject for this clinic was a man of 33 who was sent into the wards in May by Dr Waters with purpura, anemia and a greatly enlarged spleen. He had fever. The patient was thought to have splenic anemia, but in August he developed a well marked aortic systolic murmur and later a tender, painful swelling in the left groin which was considered to be an aneurysm. Osler then says,

"These features, of course, put an entirely different construction on the case' and it was evident that he had an infectious endocarditis. Cultures were made from the blood by Dr Gibson, from which have grown a streptococcus which has the character of the form described by Libman in connection with these cases. I must say that this sequence of events was quite outside my experience, but there are one or two similar cases in the literature. Only the other day Dr Parkes Weber, into whose clinical net come all sorts of peculiar and instructive cases, sent me a paper in which is described a case of malignant endocarditis, the early features of which resemble those of splenic anemia \*

After describing the autopsy at which extensive vegetations were found on the aortic valves and drawing attention again to the prominent enlargement of the spleen, he concludes by enumerating the important features of this type of infective endocarditis. They are listed as

- "1 Chronicity
- 2 Latency
- 3 Fever of the so called septic type
- 4 Embolic attacks
- 5 Ephemeral Cutaneous nodes
- 6 Blood cultures
- 7, Lastly the endocarditis is pathologically unlike the ordinary ulcerative form"

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\* British Journal of Dermatology February 1910

Though some enlargement of the spleen is common in subacute bacterial endocarditis, it is only the occasional case, and usually during the bacteria free stage, as Libman (15) has emphasized, that this organ attains very great size. The case reported by Osler is one of the earliest on record, in which great enlargement of the spleen was combined with bacteraemia.

There is no intention of reviewing the vast literature on subacute or chronic bacterial endocarditis, for this has been done recently by Libman, (15) who gives 226 references to papers on this subject. The aim of this study is to follow through Osler's contributions, his gradual realization that there was a form of infective endocarditis that could be distinguished by its symptoms, by its signs and by the duration of the illness from the acute variety of the disease. This concept culminated in his paper published in 1909, and was almost immediately substantiated by the publication of Horder's elaborate investigations. During the next year Schottmuller (16) added still further evidence to support this conclusion, by his description of cases of what he termed "Endocarditis Lenta." These, he discovered, were caused by streptococcus viridans. The disease soon became familiar in this country through the work of Libman (17, 18, 19) who, in addition to other observations reported the occurrence of cases in a so called "bacterae free stage." Loehlein (20) described in detail the form of hemorrhagic nephritis that is related to subacute bacterial endocarditis. Baehr (21, 22) made careful studies of the renal lesions in subacute bacterial endocarditis and found that a diffuse glomerular nephritis was most apt to occur in those patients who died in the bacteria free stage of the disease.

The literature on subacute bacterial endocarditis accumulated so rapidly that Debré (23) in 1919 collected sufficient material for a review of the subject which was elaborated still further by Blumer (24) in 1923, whose article forms the basis of our information on the subject up to that time. Libman stated in 1918 that he had seen 300 cases.

It seems surprising that up to this time almost no mention had been made in the general literature of the clubbed fingers that so often appear during the course of the disease. As early as 1912 Major (25) mentions this sign which was recorded in the histories of 3 of 6 cases of



subacute bacterial endocarditis amongst 15 probable cases occurring in The Johns Hopkins Hospital. He also noted that though patients might have negative blood cultures during life, culture from the vegetations on the heart valves at autopsy, yielded streptococci. Cotton (26) had noted the presence of clubbed fingers in 1920 and published a more elaborate study of this condition in his cases of subacute bacterial endocarditis in 1922 (27). In a survey of 721 cases of valvular disease occurring in Lewis' Clinic he found that 63 patients had clubbing of the fingers. Of these 63 cases, 44 or 70% had the signs and symptoms of chronic infective endocarditis. Sometime later Blumer (28) though he had omitted to mention the subject in his review, reported similar cases and in addition described the splinter-like hemorrhages beneath the finger nails.

Osler's early observations on the occurrence of infectious endocarditis in patients with malformed aortic valves, most of which he considered to be congenital defects, have already been quoted in some detail, but his paper seems to have attracted little attention. That the matter is one of importance has been pointed out by Lewis and Grant (29, 30) in their classical papers on the subject. Indeed as was shown by Abbot (7) somewhat later, other congenital malformations of the heart such as patent ductus arteriosus, as was recorded by Robinson (31) in 1905, and patent interventricular septum, may form a nidus for engrafted bacterial infections. These papers are of considerable significance in relation to the general conclusions that subacute bacterial endocarditis does not usually affect the aortic valves in syphilitic aortitis (32) although as Grant (33) has found in his elaborate analysis of 1000 men suffering from heart disease, subacute bacterial endocarditis occurred in non-syphilitic disease of the aortic valves much more frequently than in any other group, a fact which also appears in Osler's papers. In the entire lot of 1000 men, subacute bacterial endocarditis on one or another valve, was present or developed during the 10 year period in 12%.

As will be pointed out later, the realization that subacute bacterial endocarditis may be engrafted upon congenital cardiac defects is significant in relations to the introduction of operative measures in an effort to cure those cases in which a patent ductus arteriosus is affected.

It is desirable to draw particular attention to Thayer's (34, 35) ex-

tensive investigations upon infective endocarditis since these come as a sequel, so to speak, at The Johns Hopkins Hospital to the earlier studies of Osler and as Osler's experiences in Montreal formed the basis for his Gulstonian Lectures, so Thayer's review of the cases in Baltimore furnished the material for his Gibson Lectures in Edinburgh in 1930. Thayer was able to collect and analyse the records of 306 carefully studied cases of infective endocarditis that had been admitted to The Johns Hopkins Hospital during a period of 40 years, or which he had seen in private practice. To these he added 232 cases recorded in the literature, making a total of 538 cases of the disease. He classified his cases according to the etiology, and defines subacute streptococcal endocarditis as one phase of the entire group in which streptococci of all varieties were the etiological agents. This organism he found was the cause of infective endocarditis in 62.5% of the 536 recorded cases. He pointed out that *B. influenza* always, and *B. hemolytic* streptococci sometimes, gave rise to a clinical picture indistinguishable from that produced by the different strains of streptococcus viridans which organism, with these exceptions, was invariably the cause of the subacute or chronic form of bacterial endocarditis. In this latter group, pericarditis is rare compared to its incidence in acute rheumatic fever and pneumococcus endocarditis. He further made the observation that of 84 cases of the subacute variety of streptococcal endocarditis in which notes as to the condition of the teeth had been made, periodontal infection was present in 54, and that in several patients the onset of the malady could be directly traced to an operation on the facial sinuses, the tonsils or the extraction of teeth.

These latter statements are in accord with previous references to the connection of oral sepsis as a portal of entry for streptococci in subacute bacterial endocarditis, a relationship which is now known to be of great frequency and importance. This was emphasized by Weiss (36) who suggested from his study of 364 cases that foci of infection due to streptococci in the mouth and upper respiratory tract were common sources of entry for these organisms in this disease. He reported 10 cases in which a history of tonsillectomy or the extraction of a tooth closely antedated the onset of symptoms. These suspicions received unquestionable support through the investigations of O'Kell and Elliott (37). These authors found that blood cultures from 138 patients un-

dergoing operation for extraction of teeth made within 5 minutes after extraction gave a growth of streptococcus viridans in 84 instances, or in 60.9%. Of 40 cases in which there was marked gum disease, necessitating multiple extractions, there were positive cultures in 30, or 75%, and in 38 patients even without detectable gum disease, extraction of one or more teeth resulted in positive blood cultures in 12 or over 31%. The implication of these investigations is obvious. They have been confirmed (38) and have formed the basis for the necessity of prophylactic treatment in all such operative procedures, but particularly in patients with valvular heart disease. Though the sulfonamides, as will be pointed out later have been of little value, penicillin has proven very effective.

Ever since subacute bacterial endocarditis was established as a clinical entity, every conceivable method of treatment that could be devised has been employed. Only recently has any substantial benefit been accomplished. Thus White (39) states that there was only one recovery in 250 well authenticated cases treated by every means known in the Boston Hospitals during 15 years prior to 1939. Though the disease has been considered almost uniformly fatal, a few spontaneous recoveries have been reported (40). The percentage of such recoveries calculated from the reports of long series has been given as from 1% to 3% of cases (41). Libman (42) reported 4 spontaneous recoveries in a series of 150 cases, but Lichtman and Bierman (43) who collected the data on 634 cases found only 6 recoveries or an incidence of 1%.

When the sulfonamides were introduced for the treatment of infections due especially to certain gram positive bacteria, great hopes were entertained that one or another of these drugs would prove efficacious in the treatment of subacute bacterial endocarditis due to streptococcus viridans. During the first few years of these trials, cases were reported in which there was, for a time, considerable improvement, but relapse was generally the rule, and it soon became evident, that, even with such adjuvants as hirudin, introduced by Kelson and White (44, 45), or hyperthermia, the total mortality was not very greatly reduced. For instance Lichtman and Bierman (43) give in their collected statistics a recovery rate in 198 cases of 6% for chemotherapy alone, and in 88 cases a recovery rate of 16% for chemotherapy combined with hirudin or hyperthermia in some form. In a review

of these forms of chemotherapy, Hunter (46) states that "at best 10% of recoveries were reported with sulfonamides alone, although, in some small series, 25% cures occurred when fever therapy was employed in addition "

During this period of chemotherapy an entirely new and different approach to the problem of therapy was introduced This consisted in the ligation, by operative procedures, of the ductus arteriosus in patients in whom this structure was the seat of subacute bacterial endocarditis Touroff (47) who was active in this field, reported in 1940 what seemed to be a cure of 5 cases by this method The next year he published (48) the results which he had obtained in 11 cases Two patients died of operative hemorrhage Of the nine survivors 6 recovered from the infection without benefit of chemotherapy Of the 3 successful results, at least 2 patients presented evidence of involvement of the valves of the heart as well as of the patent ductus arteriosus

In this connection reference must be made to the remarkable case described much earlier by Hamman and Rienhoff (49) in which streptococcus viridans septicaemia was cured by the resection of an infected arterio-venous aneurysm between the external iliac artery and vein

What appears to be, up to the present time, the final chapter in the treatment of subacute bacterial endocarditis, came with the introduction of penicillin This antibiotic has proven to be highly effective The recent papers by Hunter(46), by Favour, Janeway and their associates (50), and by Herring and Davis (51) furnish information concerning various aspects of this important subject Hunter's results were highly successful Of 49 patients treated at the Presbyterian Hospital in New York, 90% were cured of their infection As 4 of these patients died later of causes unrelated to the infection, the actual recoveries amounted to 83% He makes the point that enormous amounts of penicillin are required to control the infection when this is caused by a relatively resistant strain of streptococci In one patient, 20,000,000 00 units a day for 16 days was required and in another patient 10,000,000 00 units in constant drip for 3 weeks Favour et al (50) record the results of treatment by penicillin in 17 cases, in 11 of which the infection was cured or arrested, and Herring and Davis (51) record 18 cases in which the infection was arrested in 13 Of

the 18 patients only 10 were alive and well, however, after one year or more. The figures given for these three series afford some idea of the differences in the number of "cures" reported by various observers. Libman (15) states that the administration of penicillin to 103 cases, reported by 6 groups of workers resulted in the cure of infection in 107 or 81.7%. Herring and Davis (51) conclude from an analysis of the literature that an average of 75% of cases have been cured by penicillin therapy.

The most comprehensive study of this entire problem has been made by Anderson and Keefer (52) who have analysed elaborately in their monograph the results of treatment by penicillin in 690 cases of Bacterial Endocarditis. Non-haemolytic streptococci were the cause of endocarditis in 457 of these patients. Arrest of the infection occurred in 282 instances or in 62% of cases. Temporary improvement occurred in 25 patients or 5.3%, the result was indeterminate in 3 or 0.7% and 147 or 32% died during treatment. The greatest number of deaths in the fatal group were due to primary infection, congestive heart failure and to cerebral embolism in order of frequency. These three conditions were responsible for death in 108 of the 147 fatalities.

It would involve an extensive discussion of the methods of administration of penicillin and the relative resistance of the infecting organism among other factors to explain why the infection was not controlled in some cases, a problem that is beyond the scope of this paper, but it is necessary to add a word concerning the fatal outcome in those patients in whom it has been shown by autopsy that the infection itself has healed completely. There appear to be at least 2 conditions that account for these deaths following recovery from the infection. One of these, as pointed out by Bloomfield (52), and considered recently by Rapoport and Ellis (53), is progressive and uncontrollable cardiac failure, due supposedly to extensive injury or destruction of one or another of the heart valves, the other is cerebral embolus. Both of these complications have been frequently noted by many writers and both at the present time appear usually to be unpredictable and unfortunately unavoidable. It has been suggested, however, that if treatment by penicillin is instituted early in the disease the danger of subsequent cardiac failure may be lessened.

It rarely happens that one can trace through scarcely more than

half a century the development of knowledge concerning a disease from its earliest definition through its recognition as a comparatively common clinical entity, which was almost uniformly fatal, to the discovery of its etiological agent or agents, with subsequent means of cure in at least 75% of cases. But so it is with subacute bacterial endocarditis. This is a disease with which Osler's name will always be associated, for his acute observations went far in establishing it as a clinical entity, and in stimulating interest in a condition that was difficult of diagnosis and practically resistant to all forms of treatment during his life time.

The sequence of events leading finally to an effective treatment of the disease, are illustrative of the astonishing advances that have been made in many fields of medicine during the last quarter of a century.

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# THE CLINICAL COURSE OF DISSEMINATED LUPUS ERYTHEMATOSUS

## AN EVALUATION OF OSLER'S CONTRIBUTIONS

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The subject of ulcerative endocarditis formed the thesis of Dr Osler's Gulstonian Lectures in 1885. He began them as follows: "It is of use from time to time to take stock, so to speak, of our knowledge of a particular disease, to see exactly where we stand in regard to it, to inquire to what conclusions the accumulated facts seem to point and to ascertain in what direction we may look for fruitful investigations in the future."

In such spirit we have undertaken this study of lupus erythematosus disseminatus, a disease to which Dr Osler contributed such brilliant thought in a series of articles on "The Visceral Complications of Erythema Exudativum Multiforme," published between 1895 and 1903 (1-3).

None of Osler's cases was studied by morphological techniques, and some may not have had disseminated lupus erythematosus. Perhaps his emphasis on the prominence of abdominal symptoms has not been borne out by subsequent experience. However, it was Osler who first emphasized the fundamental concept that the alterations occurring in the skin of patients with this disease had their counterpart in the internal organs. The clinical genius of Dr Osler could not be better exemplified than by the following description of the visceral complications of erythema exudativum multiforme which he wrote in his first article on this subject published in December, 1895, at a time when there was no knowledge of the pathological changes in this disease:

"By exudative erythema is understood a disease of unknown etiology with polymorphic skin lesions—hyperemia, edema, and hemorrhage—arthritis occasionally, and a variable number of visceral manifestations, of which the most important are gastro-intestinal crises, endocarditis, pericarditis, acute nephritis and hemorrhage from the mucous surfaces. Recurrence is a special feature of the disease and attacks may come on month after month or even throughout a long period of years. Variability in skin lesions is the rule, and a case may present in one attack the features of an angioneurotic edema, in a second of a multiforme or

THE  
VISCERAL LESIONS  
or  
THE ERYTHEMA GROUP.

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In December, 1895,† I published a series of eleven cases characterised by—

I Polymorphous skin lesions (a) acute circumscribed œdema, (b) urticaria, (c) purpura, and (d) ordinary exudative erythema

II Polymorphous visceral lesions (a) local serous or hæmorrhagic exudate in the walls of stomach or bowels, causing (1) crises of pain and (2) hæmorrhages, (b) acute nephritis, and (c) certain rare pulmonary and other lesions

III Infiltration of synovial sheaths, peri articular tissues, and arthritis

It would have been better, as some of my dermatological friends suggested, not to have described the cases under the name erythema exudativum multiforme, the term which has been used to designate the so called pure type of polymorphous erythema, but it was really very difficult to find a name under which to group the cases. Duhning has suggested that the majority of them should be regarded as purpura rather than erythema, but in only Cases 6, 7, and 11 was purpura the sole lesion, while in the remaining cases there was exudate (either serous or hæmorrhagic or both), with swelling

In the following communication I shall give the subsequent history

\* Paper prepared for the Jacobi Festschrift, New York, 1900

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nodosum erythema, and in a third those of peliosis rheumatica. The attacks may not be characterized by skin manifestations, the visceral symptoms may be present, and to the outward view the patient may have no indications whatever of erythema exudativum. Of the eleven cases here reported the visceral manifestations were as follows. In all gastro-intestinal crises, colic, usually with vomiting and diarrhea, five had acute nephritis, which in two cases was followed by general anasarca and death, hematuria was present in three cases, hemorrhage occurred from the bowels in three cases, from the stomach in two cases, from the lungs in two cases, from the nose in three cases, one patient had spongy and bleeding gums, two cases presented enlargement of the spleen, in one case there were recurring attacks of cough and bronchitis without fever, in one case there was a heart murmur. Five of the cases had swelling about and pain in the joints. The remarkable tendency to recur is a feature of all forms of exudative erythema. It will be noted that of the cases here reported in only one was the attack single. In the others there were multiple outbreaks distributed over periods ranging from two months to eight years. A remarkable circumstance, which I had not seen mentioned in the literature, is the recurrence of severe attacks without cutaneous manifestations. In the first two cases, which are at present under observation, one would not for a moment suspect the true nature of the disease from the existing manifestations, which are entirely visceral."

In his third article published in 1903, he analyzed his total series of twenty-nine cases. In addition to the visceral manifestations which he had previously described he added two cases with central nervous system symptoms, in one, active delirium at the time of recurring attacks, while in the other there were five or six attacks of aphasia and hemiplegia with cerebral and cutaneous manifestations lasting over a period of fourteen years. He states that it is not improbable that these transient attacks were due to vascular changes in the brain, the counterpart of these occurring in the skin. In describing the respiratory manifestations he cites one case in which there was protracted pneumonia following directly upon a severe attack of exudative erythema. He states that it is likely that the recurring skin lesions, the pneumonia, the general glandular enlargement, and the fatal nephritis were due to one and the same cause. In reference to the cardiac manifestations, Osler stated that he had seen only one case of acute endocarditis, but that there were a good many such instances described in the literature. Three of the cases in his series had heart murmurs and one had pericarditis occurring as a terminal event. In fourteen of his cases there was an acute nephritis, indicated by albumin and numerous tubular casts and in seven cases hematuria. The

seriousness of the nephritis was emphasized by the fact that five of the seven patients died with uremia. Arthritis or arthritic pains were present in seventeen cases. The joint lesions were slight, as a rule, and in no case was there a severe polyarthritis. In many cases there were hemorrhages from the mucous membranes, including epistaxis, blood in the stools and in the urine. In one case the final symptoms were those of severe purpura hemorrhagica after outbreaks of urticaria for many years.

Some of the details of these manifestations so clearly described by Osler have been filled in since the publication of his article, and much progress has been made in recognizing the morphological lesions characteristic of this disease. However, until some certain diagnostic method is at hand the broader aspects of its clinical manifestations will remain incomplete. Many who have subsequently reported similar cases have not pointed out the significance of Osler's contribution. In 1924, Libman and Sacks (4) described four cases under the title "A Hitherto Undescribed Form of Valvular and Mural Endocarditis." All showed the same distinctive clinical picture, two with and two without skin manifestations, and all had a form of endocarditis which they designated as atypical verrucous endocarditis. Dr. Libman stated that this demonstrated that the clinical picture first described by Kaposi (5) and amplified by later observers may occur in the absence of any eruption. This fact had been clearly pointed out some thirty years ago by Dr. Osler. The subsequent contribution of Gross (6), in describing the peculiar histological changes of so-called atypical verrucous endocarditis in the endocardium and pericardium of the majority of cases of lupus erythematosus unaccompanied by a macroscopic endocarditis, did much to clarify the whole subject. A large series of cases was presented by Baehr, Klemperer and Schiffrin (7) in 1935 under the title "A Diffuse Disease of the Peripheral Circulation (Usually Associated with Lupus Erythematosus)." They described the widespread vascular lesions and believed that the disease had a vascular origin. In 1936, Friedberg, Gross and Wallach (8) described cases under the title "Non-Bacterial Thrombotic Endocarditis Associated with Prolonged Fever, Arthritis, Inflammation of Serous Membranes and Widespread Vascular Lesions." These appear to be instances of lupus erythematosus disseminatus without skin lesions, a condition which was clearly recognized by Osler.

Klemperer, Pollack and Baehr (9) described the pathology of disseminated lupus erythematosus in 1941. They stated that the characteristic organic changes, previously considered as heterogeneous, could be understood as local manifestations of the widespread damage of collagen tissue, and that lupus erythematosus could no longer be regarded as a disease with predominant localization in a single organ or as a diffuse disease of the peripheral circulation. In a subsequent paper (10) they pointed out the fallacy of considering diseases in which alterations in collagen tissues predominate, such as rheumatic fever, disseminated lupus erythematosus, scleroderma and allergic states, as being necessarily related etiologically. They suggest that a derangement of the colloidal state of the entire collagenous tissue system may exist. Such a derangement may have a variety of causes.

#### THE CLINICAL MANIFESTATIONS OF DISSEMINATED LUPUS ERYTHEMATOSUS

It is appropriate to review the current status of the knowledge of the clinical picture of this disease syndrome. This analysis is based on a series of thirty-two cases seen in this hospital in recent years, in all of whom the diagnosis was proved by pathological examination.

It has frequently been emphasized that this disease occurs predominantly in females in the age group from ten to thirty. In the largest previously reported series, that of Baehr, Klemperer, and Schiffrin (7), there were twenty-two females and only one male. Nine of the patients were between ten and twenty years of age and nine were between twenty-one and thirty. In the present series there were ten males and twenty-two females. Twenty-five of the patients were white and seven colored. The age distribution was as follows: one to ten—1, eleven to twenty—7, twenty-one to thirty—3, thirty-one to forty—12, forty-one to fifty—5, fifty-one to sixty—2, sixty-one to seventy—2.

Of these thirty-two patients six were still living at the time of the last observation and twenty-six had died. The average duration of the disease from the onset of the first symptoms to death was four years and four months.

In view of the difficulty in making this diagnosis when the characteristic skin manifestations are not present, it is of some interest to see what the initial manifestations were in this series of patients.

Most prominent were joint manifestations which formed the initial complaint in seventeen patients. In fifteen of these there were objective findings and the other two complained of arthralgia. A characteristic skin eruption was the first finding in thirteen of the patients. In six there was unexplained fever and a complaint of weakness. Weight loss, gastro-intestinal disturbances and hemorrhagic phenomena were present in three.

If one now considers the incidence and type of the various manifestations during the entire course of the disease, the complexity of the clinical picture becomes more evident. Fever was a constant manifestation in the active stage. Several of the patients had high unexplained fever as the sole clinical manifestation, and only at autopsy was the definite cause of the fever explained. Elevation in temperature of a significant degree which could not be accounted for other than as a manifestation of this disease was recorded at some stage in thirty of the cases. Chilly sensations were frequently noted by the patient during the febrile periods.

Twenty-seven of the thirty-two patients complained of pain in many joints during the course of the illness. The joint pains were often migratory, and occurred in episodes not infrequently separated by many months or even years. In many instances the changes led to deformity, sometimes ankylosis, and quite frequently muscle atrophy and contractures. A diagnosis of typical rheumatoid arthritis was often made. Generally there were objective changes in the joints including local heat, redness, and swelling, but, occasionally, although the patients complained bitterly of arthralgia, little was found on examination.

The arthritic changes may occur early before skin or other visceral manifestations appear. This is well illustrated by the following case summaries.

*Case 1* (JHH 441345), a forty-six year old white woman, who six years before death developed pain localized to the knees, ankles, shoulders, elbows, wrists and fingers. Pain was accompanied by swelling of the involved joints with redness. These manifestations fluctuated in intensity. Three years before death she received two injections of gold which were followed by no untoward manifestations. Six months before death, at a time when she was free of any acute joint manifestations, but did have flexion contractures of both elbows and limitation of motion of the fingers, she developed anorexia and generalized fatigue. Following this

there was rapid loss of weight and the onset of an irregular fever with occasional chilly sensations. Three months before death she was admitted to a hospital for study where she was found to have a severe anemia and leukopenia for which no cause could be found. At this time she complained of intermittent episodes of severe epigastric discomfort, but radiological examination revealed no organic gastro-intestinal lesions. One month before death, on admission to this hospital, she had a temperature of 100.4, pulse rate of 110. She was very emaciated and quite weak. There was definite general glandular enlargement. Typical findings of rheumatoid arthritis with ulnar deviation of the fingers were described, and there was a sclerodermatous appearance to the skin with atrophy of the muscles surrounding the joints. There was also some generalized muscular atrophy. Shallow ulcers were seen on the soft palate. The heart was slightly enlarged to the left. The aortic second sound was accentuated, and there was a systolic murmur at the base. To the left of the sternum there was a pericardial friction rub. A protodiastolic gallop rhythm was heard at the apex. The liver edge extended two fingerbreadths below the costal margin. During the next month she had a fluctuating fever with temperature rising to 101 or 102°F each day. Four days before death she had a Jacksonian type of convulsion with deviation of the eyes to the left, winking of the lid, facial twitching and clonic movements of the left arm and leg. There were numerous such episodes from that time until her death. She became comatose, and there was paresis of the left side of the body. Laboratory examinations revealed red blood count 2.3 million, hemoglobin 8 grams, white blood count 3.7 thousand with 18% myelocytes, 6% juveniles, 19% polymorphonuclear cells, 53% lymphocytes, 4% monocytes. Stool examination showed a positive quaiac test for blood. Examination of the urine was normal. At autopsy the classical lesions of lupus erythematosus were found. Histological examination revealed hyaline necrosis of collagen with overlying fibrinous thrombus at the base of the mitral valve, foci of collagen degeneration with associated inflammatory cells in the myocardium and about the esophagus, perivascular fibrosis in the spleen and hyaline masses in many glomeruli, organized and fresh pleuritis and pericarditis, anaphylactoid type of pneumonitis, fibrinoid degeneration of the walls of the arteries in the stomach and bladder with aneurysm formation (Fig. 1), intracerebral and subdural hemorrhage in the region of the left occipital lobe (Fig. 2). *During the course of this patient's illness there were never any cutaneous lesions.*

*Case 2 (JHH 248475), a thirty-five year old colored male entered the hospital three years before death because of a polyarthritis accompanied by vomiting, chills and fever. Everyone who saw the patient made the diagnosis of rheumatoid arthritis. The white cell count was 3,440 and the hemoglobin was 11.5 grams. Differential count showed 5% myelocytes, 21% juveniles, 38% adult polymorphonuclears, 1% basophiles, 23% lymphocytes, 12% monocytes. The patient was followed in the Arthritis Clinic for many months where treatment with gold was given. It was noted at the start of treatment that the total serum protein level was 8.78 grams, with a globulin level of 5.59 grams. He received a total of 12*



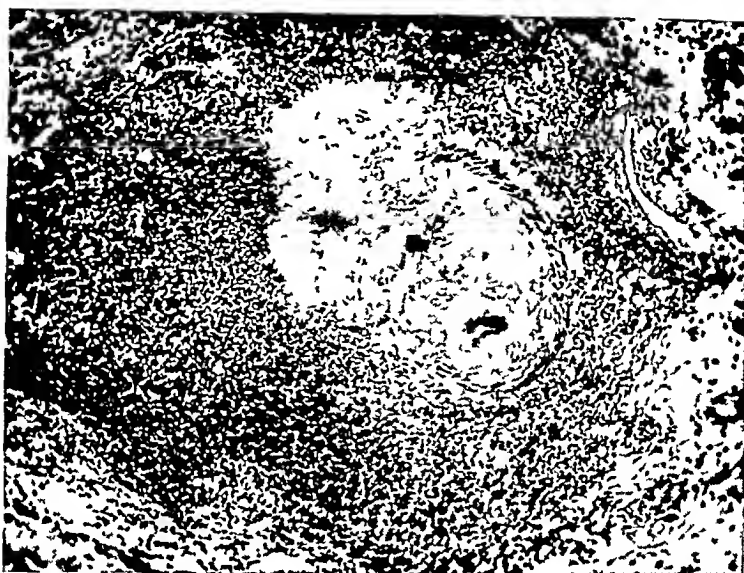


FIG 1 FIBRINOID DEGENERATION IN WALL OF A GASTRIC ARTERY WITH ANEURYSM FORMATION (CASE 1) AUTOPSY 20921



FIG 2 Intracerebral hemorrhage (Case 1) Probably secondary to lesions of the cerebral arteries similar to that seen in Figure 1 Autopsy 20921

weekly injections of gold at the end of which time his joint manifestations had subsided, and the patient did not return for observation. Five months before death he began to lose weight and noted the onset of anorexia, night sweats, pains in the ankles and wrists and bleeding from the gums. One month before death he developed pain in the left lower quadrant, had a chill and began to vomit. A short while later he developed a pleuritic type pain in the left chest. On examination three weeks before death the temperature was 99.6, pulse 120, respirations 30, blood pressure 210/155. The patient was very emaciated and looked chronically ill. There were numerous round light colored maculae over the legs. There was fusiform swelling of the phalangeal joints, limitation of motion and some deformity of both wrists. The eyelids were quite swollen, and the tissues over the eye ball were very red and edematous. Examination of the retina showed many exudates and numerous hemorrhages. The heart was slightly enlarged to the left. There was a soft apical systolic murmur. The liver and spleen were not palpable. Laboratory examinations revealed hemoglobin 13 grams, white blood count 5,900. The urine showed a specific gravity of 1020 with 4+ albumin, occasional red blood cells and white blood cells and 2 to 3 hyalin and granular casts per high power field. X-rays of the hands and feet showed the changes seen in chronic rheumatoid arthritis. Blood non-protein nitrogen was 50. Total serum protein was 8.38 with 5.07 grams of globulin. A week before death there was a very rapid increase in the size of the heart and the pulsations were noted to be rather feeble. Fluoroscopic examination revealed a cardiac outline compatible with the presence of a pericardial effusion. The patient ran an irregular fever during the three weeks before death. Ophthalmoscopic examination revealed thrombosis of the right central retinal vein. The patient continued to have attacks of abdominal pain. Autopsy examination revealed the typical lesions of lupus erythematosus. There were onion-skin lesions about the splenic arterioles. Fibrinous pericarditis was found as well as a fibrinous pleurisy. There were hemorrhages in the pleura, endocardium and quadriceps muscle.

Cutaneous lesions appeared during some phase of the illness in twenty-six patients. Weakness, weight loss and easy fatigability were frequently associated symptoms. In addition to the characteristic erythematous eruptions encountered it should be noted that in six individuals purpuric manifestations were the central feature. In two patients cutaneous pigmentation only was noted, and urticaria was the predominant type of lesion in another. It should be stressed that the lesions were not confined to the skin. In ten of the thirty-two patients ulcerative lesions of the oral mucous membranes were observed.

A significant degree of generalized lymphadenopathy was described in sixteen of the patients. In addition, there was localized glandular

enlargement in another six, usually involving the axillary and cervical glands. In the remaining ten patients no significant alteration in the size of the lymph nodes was described. Occasionally the lymphadenopathy was prominent enough to suggest the possibility of some type of lymphoma.

The analysis of the renal manifestations in this group of cases who died and came to autopsy brings out certain points of interest. In two patients there were no renal manifestations during life and no lesions of lupus erythematosus were found in the kidneys. In one instance clinical examination was entirely normal, but the classical lesions were found in the kidneys at autopsy. Twelve of the patients had an abnormal urinary sediment with abnormal renal function and the only kidney abnormalities found at autopsy were due to lupus erythematosus. In seven instances an abnormal urinary sediment was described during life but renal function tests were entirely normal, and in all instances the renal manifestations of lupus erythematosus were the only histological abnormalities found. Eight patients developed a significant degree of azotemia for which no basis could be found other than the lesions of lupus erythematosus in the kidneys. The other patient had multiple renal abscesses. Only four patients in the entire group had a significant degree of hypertension. In two of these the only renal lesions found at autopsy were those of lupus erythematosus. In one patient the kidneys were entirely normal on histological examination, and in the other the only abnormalities were those produced by arterio- and arteriolosclerosis with no changes characteristic of disseminated lupus being found.

The occurrence of pulmonary manifestations during the course of lupus erythematosus has been repeatedly emphasized. In nineteen of the thirty-two cases in the present series there were clinical evidences of pulmonary involvement at some time during their course. Clinical evidence of pneumonia was a very common finding, particularly in the terminal stages, and was often thought to be part of the picture of lupus erythematosus. However, in only three cases were the classical lesions of this disease found at autopsy. In most instances the pneumonia was lobular in distribution and of bacterial origin. In one case there was a bilateral empyema and in another a pyogenic lung abscess.

Two cases showed active pulmonary tuberculosis with cavitation. In one of the patients with pulmonary lesions due to lupus erythematosus physical examination of the chest showed nothing remarkable. X-ray study revealed a non-tuberculous type of infiltration throughout both lung fields (Fig 4). At autopsy Dr Rich reported the presence of typical lesions of lupus erythematosus in the lungs, and no other pathological abnormalities were present. In another case, examination revealed transient medium and coarse rales in various areas of both lung fields posteriorly. X-ray examination showed an increase in hilar markings spreading out into both lung fields suggesting either infection or congestive change. At autopsy the findings of "anaphylactoid or rheumatic pneumonia" were described, and there was no evidence of any bacterial invasion. In the other instance there was no suggestion on physical examination or by x-ray of any abnormality in the lungs. At autopsy very small, scattered areas were seen in which the typical lesions of lupus erythematosus disseminatus were described.

Osler pointed out that the course of the pneumonic manifestations might be very protracted. Rakov and Taylor (11) reported a case without cutaneous manifestations in which persistent signs of consolidation of the lungs were the most prominent feature of the clinical course, persisting from early after onset of symptoms until death eight months later.

Involvement of the various serous membranes has been described as a common clinical feature of this disease. Nine of these patients had a fibrinous pericarditis, while in three there was, in addition, a small effusion. The shape of the cardiac silhouette was characteristic, but the degree of effusion was never sufficient to produce any clinical evidence of cardiac tamponade. In eleven patients pleuritis was discovered at autopsy, free fluid was present in almost half of these cases, in certain instances being large in amount and bilateral. In no instance was there ascites in the absence of generalized edema. Two patients had clinical evidence of peritoneal irritation. This led to the impression in one that the patient had an acute abdominal condition requiring surgery, but operation revealed only tissue edema. Evidence of involvement of the peritoneal surfaces revealed at autopsy was

unusual except for mild perisplenitis and perihepatitis. Three patients complained of severe myalgia and in two instances this, together with muscular atrophy, led to the clinical diagnosis of dermatomyositis.

Seven of the patients at postmortem examination were found to have verrucous vegetations on one or more of the heart valves similar to those described by Libman and Sacks (4). In only one instance did these alterations of the valves seem to be associated with any impairment of cardiac function. In this individual verrucous vegetations



FIG. 3 SMALL ORGANIZING VEGETATIONS ON MITRAL VALVE  
(J. A., AUTOPSY 17346)

were found on the mitral valve which was thought to be so altered by these changes as to result in insufficiency. The left heart was dilated and enlarged and the patient showed evidences of heart failure both prior to death and at postmortem examination. The patient was seen by Dr. Louis Hamman who described enlargement of the left ventricle, the presence of a gallop rhythm, and a loud first sound in the mitral area. This was followed by a blowing systolic murmur and a sound in diastole which was thought to represent a diastolic murmur. The blood pressure was 160/95. In the other instances in which verrucous endocarditis was found at autopsy the only associated physical altera-

tion noted during life was a soft apical systolic murmur. Murmurs of this type were not infrequently noted in patients who died and whose



FIG 4 X-ray of chest (Case 6), showing extensive non-tuberculous pulmonary infiltration and enlargement of the cardiac silhouette due to pericardial effusion. Histological examination revealed focal hemorrhages and focal alveolar exudate in lungs, with organization, thought to be typical of the changes seen in rheumatic fever, periarthritis nodosa, and sulfonamide hypersensitivity (Autopsy 19429)

valves were found to be perfectly normal at autopsy. Of much greater significance, in so far as the development of cardiac insuffi-

ciency was concerned, was the presence of myocardial scarring secondary to arteritis and ischemic necrosis of the myocardium. Of interest is one patient who at autopsy was found to have verrucous vegetations of the mitral, aortic, and tricuspid valves (Fig 3). Streptococci were found imbedded in the verrucous vegetations involving the tricuspid valve. It was thought that this patient had a secondary bacterial endocarditis establishing itself in a valve previously involved by the endocarditis of disseminated lupus erythematosus.

In the series of cases reported by Osler, gastro-intestinal manifestations were emphasized. Since that time, in the cases described in the literature such manifestations have been insignificant in most instances. This has led some authors to the conclusion that most of the patients described by Osler did not have lupus erythematosus disseminatus. In the present series five patients had a significant degree of diarrhea. In four of these the stools were bloody, and one had a massive hemorrhage. In four cramping abdominal pain was a prominent symptom. In other patients extensive lesions of classical lupus erythematosus have been found in the vessels of the gastro-intestinal tract. The following case illustrates the occasional prominence of abdominal manifestations in the clinical picture.

*Case 3* (JHH 227999), a forty-five year old white male who developed unexplained fever and diarrhea in October, 1940. After a few days there was spontaneous remission, but he continued to have bouts of fever and diarrhea throughout the winter. Stool examinations and x-ray studies were done, but no basis for his complaints could be demonstrated. In March, 1941 fever and diarrhea returned with severe epigastric discomfort. The following month his temperature rose to 103° to 105°F each day for short periods of time interspersed with periods of normal temperature. During the course of the fever he complained of chilly sensations and developed "lumps in the muscles." In May, 1941 he developed an erythematous eruption which was most prominent over the "butterfly area" of the face, and he was found to have both a pleural and pericardial effusion. Renal impairment was evidenced by albumin, white cells, and red cells in the urine and a non-protein nitrogen level of 59 mgm %. His condition grew rapidly more critical, and he died on May 17, 1941. At autopsy the classical lesions of lupus erythematosus disseminatus were found with endocarditis, hyaline masses in the glomerular loops, and perivascular fibrosis of the splenic arteries. There was a bilateral hydrothorax and a fibrinous pericarditis. Figure 1 shows the type of vascular lesions which are found in the gastro-intestinal tract. The clinical manifestations of this patient are described above (Case 1).

Enlargement of the liver to a significant degree was noted frequently both clinically and at postmortem examination. This enlargement was due in most instances to the deposition of large amounts of fat. Occasionally widespread necrosis of liver tissue was observed. It is interesting that in only one instance was jaundice noted in this group of patients, and this was of the obstructive type. This patient had generalized edema with ascites, and the jaundice was due to edema of the biliary ducts. In only a small number of patients was liver function adequately studied. In these cases there was evidence of diffuse parenchymatous disease manifested by elevation of the cephalin flocculation and the thymol turbidity. These tests were abnormal even in instances in which the albumin-globulin ratio was not altered. In no instance, however, were the lesions of lupus erythematosus disseminatus seen in histological examination of the liver. Focal central necrosis and excess fat were present in six of the cases described by Coburn and Moore (12). The nature of these changes deserves further study.

A palpable spleen was noted in less than half of the cases, and the enlargement was only moderate in those instances. Kaposi (5) reported an instance of acute lupus erythematosus in a female aged 15, the skin manifestations making their appearance about eight days after the onset of purpura hemorrhagica. Osler also recognized the occurrence of purpura hemorrhagica in the series of cases which he described. Too much significance, however, cannot be attached to these observations as at that time the hematological criteria for the diagnosis of thrombocytopenic purpura were not established. In 1933, Lyon (13) described a twelve year old boy who had thrombocytopenic purpura with leukopenia. Subsequently, the patient developed the typical skin lesions of acute lupus erythematosus. In 1937, Keil (14) described the case of an eighteen year old white female who developed thrombocytopenic purpura. The patient had a satisfactory response to splenectomy. However, eighteen months later she returned to the hospital with the characteristic skin manifestations of lupus erythematosus. The patient died almost seven years after her first appearance, apparently in uremia. Jones and Tocantins (15) reported the case of a female patient who developed lupus erythema-



tosus several months after splenectomy for thrombocytopenic purpura, at a time when the platelet count was restored to normal

In the present series of cases thrombocytopenic purpura occurred in four and in all of them the characteristic lesion of periarterial fibrosis was found in the spleen on histological examination

The following case bears certain similarities to those previously reported in the literature

*Case 4* (JHH 274052), a thirty-nine year old white female who for a period of eleven years had had a recurrent eruption over the face each summer following exposure to the sun. Three years before death she had developed severe metrorrhagia and gave a history of easy bruising. At the time of examination there were numerous purpuric spots and ecchymoses on the skin. Examination of the blood showed a hemoglobin of 9.8 grams, white cell count of 3050 and a platelet count of 41,000. The differential white cell count was normal. The patient was seen by Dr. Wintrobe who made a diagnosis of thrombocytopenic purpura. During observation over a period of several months there was no improvement, the platelets remaining between 30,000 and 50,000 with the bleeding time ranging from 8 to 14 minutes. Bone marrow examination showed no platelet-producing megakaryocytes present. A splenectomy was done and review of the sections by Dr. Arnold Rich revealed the typical lesions of lupus erythematosus. Following operation her white cell count rose to 19,800 and the platelet count to 150,000. The bleeding time returned to normal, and the menstrual flow became normal in amount. During the next three years, with the exception of the facial eruption during the summer months, the patient felt quite well. Thirty-six hours after the final admission she developed anorexia and fever accompanied by pain in the muscles, joints, and skin. At the time of admission to the hospital she was acutely ill with a high fever. The skin was warm and moist, and there were numerous purpuric spots over the malar eminences. There was an erythematous blush over the chest and abdomen. The following day the purpuric eruption over the bridge of the nose was found to be even more marked, and the erythema over the chest had developed a dark purplish color. Blood culture and spinal fluid culture revealed type 12 pneumococcus. The patient was in severe peripheral circulatory collapse and died within forty-eight hours of her admission to the hospital. Examination of the blood showed a hemoglobin of 14.5 grams with 12,600 white cells. The platelet count was 42,000.

A second patient has had no further bleeding tendency for eight years following splenectomy for thrombocytopenic purpura and has developed no manifestations of disseminated lupus erythematosus, although histological examination of the spleen showed the characteristic type of periarterial fibrosis.

*Case 5* (JHH 207012), a twenty-four year old white female who entered the hospital in July, 1940 complaining of the appearance of purpuric spots on the legs, thighs and buttocks over a period of five weeks. Examination at that time showed numerous petechiae over the entire body, small ulcerations on the lower lip, bleeding gums and hemorrhage from the nose. Hemoglobin was 11.2 grams, white cell count 6,250 with normal differential. The bleeding time was greater than fourteen minutes. Tourniquet test was strongly positive. Platelet count was 50,000. After operation the platelet count did not rise until the fourth day when it reached 300,000. At that time bleeding had ceased, and the bleeding time was one minute. The clot which did not retract after four days prior to operation now began to show retraction in one hour which was complete in twelve hours. The spleen showed hyperplasia of the malpighian bodies as well as numerous lesions of periarterial fibrosis described by Dr. Rich as characteristic of those seen in lupus erythematosus disseminatus.

The most that can be said of this group of cases is that there was thrombocytopenic purpura, and that the characteristic lesions of lupus erythematosus were found in the spleen.

The presence of anemia in this condition has been emphasized. Fourteen patients in this series developed a moderate degree of anemia, and in an additional eleven it was progressive in severity. In the remaining seven no depression of the erythron was noted at any time during the course of the illness. Thrombocytopenia has been regarded by some as a characteristic feature of disseminated lupus. Only five of these thirty-two patients showed an appreciable reduction in the number of circulating platelets. Four of these patients had the clinical picture of thrombocytopenic purpura as mentioned above. Leukopenia has also been described as a very frequently encountered alteration in the blood picture of patients with this disease. Eighteen of the group showed at some time during the course of their illness a total white cell count of less than 5,000 per cu m. Attention should be called to the fact that when patients with a significant degree of leukopenia acquired a secondary bacterial infection, leucocytosis developed with counts rising as high as 25,000 per cu m in some instances.

Nervous system manifestations which were directly attributable to the lesions of lupus erythematosus formed an important feature of the clinical picture in many of these patients. In four, convulsive episodes of a generalized nature were described. In an equal number

there was a toxic psychosis which necessitated care in a psychiatric hospital in two instances. Three patients had transient periods of coma with no basis other than the disease under discussion. In an equal number of patients a hemiplegia (see Case 1) developed. In two others there were transient episodes of bilateral ptosis, and in one repeated attacks of Jacksonian epilepsy (see Case 1). In several instances there were delirium, coma or convulsive episodes probably associated with uremia, no other lesions being found in the kidneys than those of disseminated lupus.

The following case illustrates the type of psychotic behavior which may develop during the course of this disease.

*Case 6* (JHH 345215), a forty-five year old white female developed an eruption over the malar surfaces of the face and generalized arthritis in August, 1944. At times there was some heat, redness and swelling of the joints, but these were never conspicuous. She lost strength rapidly, and edema of the ankles appeared. In December, 1944 she developed very erratic behavior, wandering off for several days at a time without informing her family of her whereabouts. This abnormal behavior was periodic up until the time of her admission on March 2, 1945. At that time there was a classical erythematous eruption of lupus erythematosus over the malar eminences. There was definite peri-orbital edema, and the conjunctivae were injected. Small lymph nodes were palpable in the axillary and inguinal regions. There was a gallop rhythm over the entire precordium, and a loud systolic murmur was heard over the base. There was pitting edema up to the region of the mid-thighs. Laboratory examinations revealed hemoglobin 10.5 grams, white cell count of 4,550. The urine contained 4+ albumin and an occasional red cell and white cell as well as granular cast. Electrocardiogram showed some prolongation of the auriculo-ventricular conduction time. She was extremely restless. Feeding was difficult because of her inaccessibility. She soon became entirely unresponsive, tossing and turning in bed so that restraining sheets were necessary. Occasionally, she would make a cry, wordless and animal-like. Choreiform movements were described. Tube feeding was attempted, but was very difficult because hemorrhages occurred when the tube was inserted. The patient never regained consciousness. Terminally, the non-protein nitrogen rose to 70 mgm %, but during the major period of the delirium it was never higher than 32 mgm %. At autopsy the patient was found to have lupus erythematosus with verrucous endocarditis, periarterial fibrosis in the spleen and classical lesions in the kidneys. There were focal hemorrhages and focal areas of alveolar exudation in the lungs with organization. Periarteritis nodosa-like lesions were described in the ovaries and tubes. Bilateral pleural effusions and ascites were noted. Numerous old and fresh petechial hemorrhages were found throughout the brain substance.

In 1940 Maumenee (16) described the retinal lesions in four patients with acute disseminate lupus erythematosus and of one patient with the chronic type. He noted the following typical lesions (1) small fluffy white spots located in the superficial layers of the retina never

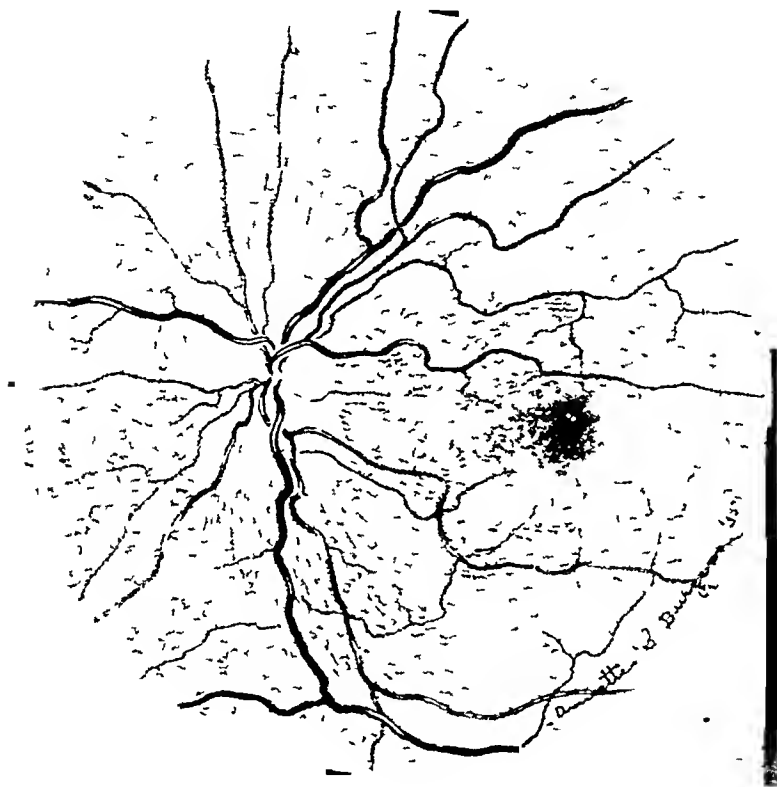


FIG 5 TYPICAL CYTOID BODIES IN THE RETINA, CONFIRMED BY HISTOLOGICAL EXAMINATION (M O, JHH 57321)

larger than the disc and usually in the posterior part of the fundus. These were very similar in appearance to the exudates seen in hypertensive retinopathy. Histologically they were all shown to be areas of cytooid bodies, (2) small hemorrhages placed superficially in the retina and not located in relation to the white patches nor to the larger retinal vessels. Microscopically these hemorrhages were found to lie

in the nerve fiber layer of the retina (3) Slight papilledema In seven of the cases in the present series the typical cytoid bodies (Fig 5) and superficial retinal hemorrhages have been seen In one instance the significance of these lesions was recognized by the Ophthalmological Consultant in a patient who had unexplained fever, splenomegaly and repeated epistaxes At a later date the classical features including the typical cutaneous eruption appeared One patient in this series developed thrombosis of the central retinal vein Thus, among patients with disseminated lupus erythematosus, lesions in the retina occur fairly frequently These lesions are not specific, but in instances in which there is no hypertensive vascular disease they may furnish a very important clue, particularly when the other visceral manifestations are not accompanied by the cutaneous lesions It seems possible that from more detailed study of the retina in a larger number of cases, with more accurate description of the nature and course of the lesions, a reasonably characteristic and diagnostic ophthalmoscopic picture might evolve

It has been pointed out that the skin lesions in this disease frequently appear after exposure to sun light This photo-sensitivity has been evident in many of the cases in this series In one patient typical "butterfly lesions" appeared over the face each successive summer following exposure to the sun over a period of twelve years (Case 4) Rich (17) has recently pointed out that disseminated lupus erythematosus, in common with rheumatic fever and periarteritis nodosa, presents a rather impressive variety of lesions each of which occurs in anaphylactoid reactions of the serum sickness type, such as fever, urticaria and erythematous skin eruptions, purpura, arthritis, necrotizing inflammatory arterial lesions, focal collagen degeneration, focal necroses of lymph nodes and of spleen, myocarditis, valvulitis, sterile inflammation of serous membranes, sterile pneumonitis and transient paresis Clinically it has not been possible to demonstrate hypersensitivity to any given substance in cases of lupus erythematosus disseminatus which might be considered as a causative factor However, the following case is of interest in this regard

*Case 7 (JHH A-30617)* This twelve year old white boy developed pains in the wrists and shoulders in 1942 at which time he was discovered to have a markedly elevated sedimentation rate When first examined in June, 1943 he had

fever and still complained of joint pains. Physical Examination at that time showed temperature  $102^{\circ}\text{F}$ , pulse 115, respirations 20, blood pressure 120/75. There were no other abnormalities. The sedimentation rate was 29 mm/hr corrected. During the next few weeks he ran a persistent elevation in temperature, complained of joint pains and developed general glandular enlargement. In December, 1943 a shower of purpuric spots occurred over his lower extremities and the palms of his hands. After that time crops of petechiae appeared at intervals, and he had frequent nosebleeds. Early in January he developed gross hematuria and slightly bluish non-tender nodules on the palms of the hands, on the elbows, and on the feet. These would develop and disappear within the course



FIG. 6 Note the characteristic distribution of the facial lesions and the evidence of oral hemorrhage (Case 7)

of thirty-six hours. One of these nodules was biopsied and showed the classical lesions of lupus erythematosus. The patient was found to have a marked reaction to a beta streptococcus antigen. Two doses of 0.1 cc of a special streptococcus vaccine were given intracutaneously at a time when the previously described manifestations were quiescent. Following the second dose his temperature rose to  $40^{\circ}\text{C}$  and numerous nodules appeared beneath the skin, first being noted six hours after the injection, he developed generalized pruritis and a diffuse erythematous eruption. Scattered showers of petechiae appeared with increasing frequency. The temperature remained markedly elevated and a few days later he vomited 400 cc of blood. Four days after the first injection he developed a typical "butterfly rash" over the malar eminences which was purplish red, slightly raised and sharply outlined (Fig. 6). On March 5 he became comatose, a positive Babin-

skin was demonstrated on the right side with flaccid paralysis of the right arm and stiffness of the left. Numerous flame-shaped hemorrhages were found in the retina of both eyes. At autopsy there were typical wire-loop lesions in the glomeruli and sections of the spleen showed scattered periarterial fibrosis of the onion skin type. No other lesions characteristic of lupus erythematosus were described.

Coburn and Moore (12) pointed out that hyperglobulinemia is a constant characteristic of the lupus state. Electrophoretic analysis showed that the increase in globulin was chiefly in the gamma globulin fraction. Autopsy study of their patients yielded no evidence that chronic infection was responsible for the production of this protein unless one considered the presence of mild infections of the bronchial tree as of significance. They state that it has been pointed out that the arterial and renal lesions of the lupus state may be referable to the deposition of coagulable material resembling serum protein, and add that "it seems likely that such physical changes may give rise to the vascular phenomena observed during life." They further state, "that disseminated lupus appears to be a disease of disturbed cellular metabolism, perhaps associated with the presence of this reactive protein."

Determinations of serum protein content were made in 22 cases of the present series. The amount of globulin present was greater than three grams per cent in 19, the average value being 4.3. The maximum values were 8.5 for total protein and 5.3 for globulin, while the respective minimum values were 3.94 and 2.06. Those of Coburn and Moore were maximum 8.7 and 5.7, minimum 4.2 and 2.1. Their mean globulin value was 3.9. In general, there seemed to be, in the present series of cases, a tendency for the patients showing the most marked arthritic manifestations to have the highest values for serum globulin. In those cases without joint manifestations the mean value for serum globulin was only 3.27. This is of interest in view of the fact that in patients with rheumatoid arthritis there is frequently an elevation of the globulin fraction with reversal of the A/G ratio.

It is relatively easy to make the diagnosis of disseminated lupus erythematosus when the patient shows all of the characteristic manifestations including fever, arthritis, cutaneous lesions, sterile effusion in serous cavities, and a picture of chronic nephritis. However, it must be emphasized that the course of the disease is characterized

by fluctuations in the degree of activity which may be great at times followed by periods of relative freedom from symptoms. In the recrudescences the active lesions may be confined to certain restricted areas. For instance, in one attack the only manifestations may be fever and a pleural effusion, while in another the important clinical abnormality may be pericarditis with effusion. In still another the cutaneous manifestations may be quite prominent. In this way the full picture of the disease only unfolds over a period of years, and the patient may be seen in several exacerbations before enough perspec-

TABLE I

*The Incidence of Complicating Diseases in 32 Cases Of Lupus Erythematosus Disseminatus*

Pulmonary tuberculosis	2
Miliary tuberculosis	2
Tuberculous adenitis	3
Tuberculosis of spine with psoas abscess	1
Pulmonary infarction	1
Lobular pneumonia	8
Type 2 pneumococcal lobar pneumonia	1
Type 23 pneumococcal lobar pneumonia with bacteremia	1
Type 6 pneumococcal lobar pneumonia with bilateral empyema	1
Type 12 pneumococcal meningitis	1
Aspiration pneumonia	2
Staphylococcic pneumonia	1
Beta hemolytic streptococcus septicemia	2
Dural Meningioma	1
Bacterial Endocarditis	1
Multiple renal abscesses with bacteremia	2
Purulent lung abscess	1
Tubo-ovarian abscess, ruptured, with generalized peritonitis	1

tive can be gained to make the diagnosis obvious. Once the diagnosis becomes clear the tendency may be to attribute any subsequent illness to the bizarre and variable clinical manifestations of disseminated lupus erythematosus. The involvement of any given organ or location may simulate a variety of other diseases affecting the same region. It is important to emphasize that any new clinical development occurring during the course of this disease must be analyzed with a completely open mind. An illness entirely unrelated may develop, be unsuspected, and lead to death. This situation becomes all the more important when one realizes that disseminated lupus erythematosus



may have long periods of remission, some patients living for ten to fifteen years or more, with intervals of complete or relatively complete freedom from active symptoms. The accompanying table (Table I) illustrates the variety of complicating diseases which may be seen during the course of lupus erythematosus. The following case report illustrates the difficulty of recognizing these concomitant diseases, and the equal difficulty of evaluating their relative importance in the overall clinical picture.

*Case 8* (JHH 260256), a 52 year old white female entered the hospital May 25, 1942 complaining of fever and arthritis of eleven weeks duration. Eighteen months previously she had developed pain and swelling in the hands and forearm which lasted for three months. There was a recurrence of the joint manifestations in March, 1942. The following month examination showed a temperature of 101.4°F, skin and mucous membranes showed mild pallor, and there was symmetrical edema of the hands and feet. The remainder of the examination was not remarkable, except for the leucocyte count of 3,600. She continued to run an irregular fever and developed crisp, inspiratory rales at both lung bases and some pleural pain at the left base. During the next few weeks she became increasingly weak and developed generalized aches and pains necessitating her admission to the hospital on May 25 for further study. Physical examination showed a mild erythematous eruption over the bridge of the nose and the cheeks. The only additional finding was a sustained ankle clonus. The patient continued to run a daily elevation in temperature from 101° to 102°. There was a heavy growth of *E. coli* in the urine. This infection was resistant to all forms of treatment available, including sulfonamides and mandelic acid therapy. Intravenous pyelograms showed both kidneys to be normal in size, shape and position. On June 29 physical findings indicated a left pleural effusion. Five hundred cml of relatively acellular fluid was removed. On June 3, there was an exacerbation of the cutaneous eruption following a mild exposure to the sun. On August 12, she developed auricular flutter and x-ray of the chest showed enlargement in the region of the left ventricle. On August 31, the patient had the first of a series of convulsions which were tonic and later clonic, being accompanied by aphasia. These occurred at increasingly frequent intervals during the next few days. The patient became much weaker and died on September 5. The final anatomical diagnosis was lupus erythematosus disseminatus, pericardial effusion, organizing perisplenitis, onion-skin lesions in the spleen, minute area of valvulitis, mitral valve, right pleural adhesions, subacute ulcerative and diphtheric cystitis and urethritis, acute pyelonephritis, right kidney, septicemia, *E. coli*, acute interstitial myocarditis, aspiration pneumonia, fatty liver, history of convulsions, dural meningioma, left parietal lobe.

In this instance the extent and importance of the urinary tract infection was not appreciated, as it was thought to be confined to the bladder. Furthermore, the fever and other manifestations to which this may have contributed were regarded as part of the picture of rapidly advancing lupus erythematosus. Convulsions are a relatively common feature during the course of lupus erythematosus and the presence of a dural meningioma in this case was entirely unsuspected.

An analogous situation existed in Case 4 described above. The final fulminating illness was thought to be due to an acute exacerbation of the disseminated lupus, principally because of the dramatic extension of the cutaneous lesions. Further study, however, revealed that a pneumococcal bacteremia and meningitis was the cause of her final acute illness leading to death.

#### SUMMARY

It seems evident from this study that Osler constructed a framework for the clinical picture of this disease, and that others have supplied the details. There seems to be little or nothing of real significance, as far as the clinical manifestations are concerned, which he omitted. The frequency distribution of the various features which he emphasized remains essentially the same, with the possible exception of the gastro-intestinal manifestations. He focused attention upon those features of the disease which, as this review makes obvious, are still of paramount importance.

- 1 The cutaneous lesions have visceral counterparts which may occur in the absence of any dermal abnormalities.

- 2 While the disease may manifest itself through alterations in any of the organ systems, the cutaneous, joint, and renal manifestations are most prominent.

- 3 Osler noted that the endocardium was at times involved, and pointed out the occurrence of hemorrhagic manifestations.

- 4 He emphasized the fact that the disease may have a protracted course, during which varied clinical abnormalities appear, rendering diagnosis difficult unless one has the opportunity to follow the course of the illness over a long period of time.

- 5 Osler realized the kinship of the diseases of the rheumatic group,

but pointed out the possibility of different etiologies. This thought finds later expression in the belief of Klemperer, Pollack and Baehr (10) that these are clinical entities of varying etiology involving primarily the same "system" of the body, thus, assuming an outward similarity, though etiologically distinct.

6 Just as certain features of the cases in Osler's series were particularly striking, in the group reported here one is impressed by the involvement of the nervous system, the similarity of the joint lesions to those of rheumatoid arthritis, the incidence of thrombocytopenic purpura, and the frequency with which secondary infections have occurred. The difficulty of recognizing these latter complications, in certain instances, in a disease with such widespread lesions is obvious. Equally obvious is the importance of their recognition in the presence of a disease which may run a relatively benign course for many years.

This review has added nothing new to the fundamental expressions of Osler. Perhaps it may serve as an echo of his clinical genius.

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CHRONIC CYANOSIS, WITH POLYCYTHÆMIA AND ENLARGED  
SPLEEN A NEW CLINICAL ENTITY

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THE group of cases here reported, with those collected from the literature, are worthy of careful study, as we have here in all probability "a definite clinical entity and one which is new to medical science," to use the words of Saundby and Russell in describing their case. The condition is characterized by chronic cyanosis, polycythæmia, and moderate enlargement of the spleen. The chief symptoms have been weakness, prostration, constipation, headache, and vertigo. A further analysis will be reserved until after the consideration of the cases.

*CASE I Cyanosis for years, of unknown origin, albuminuria, rapid pulse, polycythæmia, high vascular tension*—Dr K, aged forty four years, consulted me October 28, 1901, complaining of a rapid pulse and diffuse cyanosis. He has been a very healthy man, active and vigorous, of good habits, has had no serious illnesses. He has been uneasy about himself, as he had detected a trace of albumin in the urine. For several years his wife has noticed that he has had a very congested appearance, and the eyes would often be deeply suffused. I have seen him at intervals for the past five years and have known him to be a very blue-faced man. He has been of a constipated habit. His eyes are somewhat prominent, but his wife says this is natural to him. He has constantly a feeling of fulness in the head, sometimes a sensation of vertigo, and for these symptoms he consulted me.

He was a well built, well nourished man, the face much suffused, the ears looked a little blue, the conjunctivæ were injected, and the lips distinctly cyanotic. The tongue also looked cyanotic. The general surface of the skin looked suffused and the anæmia left after pressure of the hand on the skin was very marked and very slowly

# OSLER'S CHRONIC CYANOTIC POLYCYTHEMIA WITH SPLENOMEGALY

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Although the concept that patients might be overburdened with blood was held by Galen and his predecessors as well as by those who followed him, as late as 1889 the hematologist, Hayem, stated that he did not believe in the existence of a plethoric state which could be ascribed to an increase of red cells (1) This was the authoritative opinion even though transitory hyperglobulia had been noted by Vogel in 1854 and polycythemia in association with chronic heart disease had been observed by Malassez and by Naunyn in 1872 (2) In 1890 Vialt (3) found high blood counts in persons living at high altitudes

In writing in 1892 "Sur une forme spéciale de cyanose s'accompagnant d'hyperglobulie excessive et persistante", Vaquez (4) attributed the condition to congenital heart disease even though there were no auscultatory signs He faced the criticism of Hayem in stating that functional hyperactivity of the hematopoietic organs was suggested by the exaggerated volume of the liver and spleen in his patient and was the cause of the increase in red cells, hemoglobin and leucocytes

Failure to find evidence of congenital heart disease at autopsy made Vaquez's case more mysterious The unique character of such cases was recognized by Cabot (1899) (5), by McKeen (1901) (6) and by Saundby and Russell (1902) (7) but it was only the last named who appreciated the fact that they were dealing with a new clinical entity Previously, in 1889 Cuffer and Sollier (8) had described the disorder very adequately but they did not examine the blood

By the time Osler's report was given it was becoming clear that there are two classes of polyglobulism relative, in which the condition is due to a diminution in the quantity of the plasma of the blood, and true, in which there is an actual increase in the number of blood corpuscles Vaquez and his pupil, Quiserne (9), in 1902 had clearly defined true polycythemia and included the disease first described by Vaquez under this head together with conditions in which there is difficulty in proper aeration of the blood, as in high altitudes, or in heart disease, congenital and otherwise

Osler gave his report before the Association of American Physicians in May, 1903. This was subsequently published in the American Journal of the Medical Sciences (10), of which the first page is reproduced here. Osler gave accent to the supposition of Saundby and Russell that the condition is a definite clinical entity and clearly described the clinical picture.

Osler presented four cases. The first patient was a physician aged forty-four years, seen by him in 1901, who for years had had cyanosis of unknown origin. The patient had detected albuminuria himself. In addition the pulse was rapid (120), there was polycythemia and "high vascular tension" (175-203 mm). The edge of the spleen was palpable. There were no signs of emphysema and the heart was of normal size with clear sounds. Osler suspected "chronic degeneration of the kidneys, with slight arteriosclerosis". The laboratory work, performed by Dr. Fitcher, revealed a few hyaline and finely granular casts in addition to the albuminuria. The red blood corpuscles numbered 9,952,000, the hemoglobin was 120 per cent (Fleischl). Several counts were made as it was thought that there might have been a mistake. The "relative scarcity of leucocytes" (4,000) was another puzzling feature.

The second patient was a Russian Jew, 35 years of age, first admitted in 1900, who complained of recurring attacks of nausea and vomiting, pain in the left side and severe constipation. Persistent and marked cyanosis of the face, hands and mucous membranes, red cell counts ranging between 6,500,000 and 10,200,000, hemoglobins of 102 to 125 per cent and leucocyte counts of 8,600 to 30,000, as well as a blood specific gravity of 1.068 to 1.083 were the chief features. It was noted that the cyanosis persisted even when there was very little vomiting.

The third patient was seen at the Lakeside Hospital in Cleveland with Dr. Lowman. This was a woman of 44 years, of English descent, who was unusually cyanosed and had an enlarged spleen. A double pterygium and failing vision had brought her to the ophthalmological division of the hospital but persistent headache attracted the attention of the internists. In this, as in the preceding cases, it was noted that the impression made on the skin with the finger disappeared slowly. The spleen extended 7.5 cm. below the costal margin. The poly-

cythemia was even more pronounced than in the other two cases 11,616,000 The leucocytes were 5,100 Sodium nitrite brought relief of the headache

The fourth patient was not seen by Osler but he suggested the diagnosis after discussion with Dr Lyon concerning Dr Stockton's case in Buffalo This was a Turkish Jew of 46 years, a shoemaker, whose chief complaints were general weakness, chronic headache, pain in the feet and legs made worse by walking, general diffuse pains in the abdomen, pain over the region of the heart, moderate chronic constipation, a slight cough and occasional attacks of shortness of breath A deepening color of the skin had been noted first four years before The red corpuscles, first counted on the day of death, were found to be 8,250,000 "The depth of color and darkness of the blood was far beyond the range of estimation for hemoglobin by the color scales of the various hemoglobinometers" The patient died "in collapse and after a few hours of drowsiness deepening into semiconsciousness" At autopsy the spleen, which had not been felt during life and which on percussion seemed smaller than normal, was found to be moderately enlarged There was only moderate emphysema in the lungs, the heart was about normal and nothing definite was found to account for the condition

It is of interest that the four cases reported by Osler typify so well the picture of polycythemia vera the incidence in middle or late life, the somewhat greater frequency in males, the high incidence in Jews, the presence of symptoms such as headache, vertigo, constipation, weakness and vascular disturbances, the finding of splenomegaly in many cases, and the occurrence of leucocytosis in some cases in addition to polycythemia

Analysis of his own and of five cases reported in the literature led Osler to no conclusion concerning the nature of the disease While the clinical picture was certainly very distinctive, the symptoms, he pointed out, were somewhat indefinite and the pathology quite obscure He suggested that a careful study be made of all forms of chronic cyanosis with polycythemia, particularly of those associated with heart disease and emphysema There are essentially only two conditions, he remarked, other than the entity under discussion which permit patients to walk into the hospital or into the consulting room in spite



of the presence of extreme cyanosis, namely congenital heart disease and emphysema. Osler recommended more accurate study of the blood, measurement of the blood volume, its viscosity and specific gravity, the amount of hemoglobin and the diameter of the red corpuscles. He was inclined to attribute the cyanosis to an increased viscosity of the blood with resulting difficulty of flow. He raised the question whether the splenomegaly might not be simply the effect of chronic passive congestion. He closed with the statement that future investigation should determine whether one were dealing here in reality with a new disease.

In Osler's second paper, which appeared in the *British Medical Journal* (11), the "Chronic Cyanotic Polycythaemia with Enlarged Spleen" in which he had become interested was distinguished from that caused by primary tuberculosis of the spleen. Several observers had pointed out that cyanosis and polycythemia may be associated with this rare form of tuberculosis. Osler called attention to the chronic course of true polycythemia and the failure to demonstrate caseous masses or miliary tubercles in the enlarged spleens in the three cases of his series in which autopsy had been performed.

The clinical lecture on "Erythraemia (polycythemia with cyanosis—*maladie de Vaquez*)", delivered at the Radcliffe Infirmary, Oxford, on November 28, 1907, and published in the *Lancet* of January 18, 1908 (12) furnished a comprehensive picture of the disease which has not been amplified to any significant degree since that time. By 1908 reports had appeared not only in France, the United States and the British Isles but also from Vienna, Wiesbaden, Budapest, Berlin and elsewhere. At least 70 cases were on record.

Osler made quite clear that priority of description rested with Vaquez and was inclined to favor the use of the name, erythremia, for the disorder following the suggestion of Turk (13) of Vienna. He discussed the "torpor, mental and physical" which characterize these patients and he noted that the first patient he had seen in the hot summer days would be "red as a rose" and look bursting with blood, while in the winter he became "as blue as indigo." Osler was very much impressed with the degree of vasomotor instability seen in many cases. He stated that "high blood pressure is the rule" and sclerosis of the superficial arteries and a trace of albumin are frequently noted, and

that attacks of bronchitis and of asthma had been described Hemorrhages, sometimes petechial, or hemoptysis, hematemesis or hematuria, had occurred in a number of cases Death from cerebral hemorrhage had been noted in several instances

The analogy with leukemia was drawn but, curiously enough, not on account of the changes in the leucocytes which may be so striking in some cases of polycythemia vera but rather because of the great increase in blood volume which Haldane had noted in cases of this disease and which Osler assumed takes place also in leukemia

In further considering the pathology of "this interesting affection," Osler pointed out that the suggestion that the polyglobulism is due to retarded destruction cannot be considered seriously since there are no clinical or anatomical facts in support of such a view The intense hyperplasia of the bone marrow was noted and by this time Osler accepted the view that the spleen participates actively in the process, for histological studies had not indicated that the enlargement was due to the accumulation of the products of hemolysis He remarked, "Nothing is more certain—in the microcosm as in the macrocosm, given a demand and there is soon a supply But here is a condition in which so far as we know there is an oversupply without any corresponding demand and the same riddle confronts us as in leukemia and several other diseases of which overproduction of a normal tissue or element is the essence" Osler discussed the suggestion made by Korányi and Bence that the disease is due to a lessened power of the red blood corpuscles to absorb oxygen, as well as Saundby's proposal that "there is such a state of capillary dilatation with slowing of the blood current that each little boatlet of blood cannot discharge its proper cargo and to make up for this failure more are put into circulation, the antithesis of the condition existing at high altitudes when as each little boatlet cannot get a sufficient cargo of oxygen in the space of time it remains in the lung capillaries, three are sent out to do the work for which two usually suffice "

Repeated bleedings were recommended by Osler for the fullness of the head and vertigo Therapeutic procedures which he thought worthy of trial included the inhalation of oxygen and irradiation over the spleen

In relation to Osler's comment concerning the blood pressure in poly-

cythemia, note may be made of the subsequent description by Gaisbock (14) of a group of cases in which hypertension was present, as well as albuminuria, in the absence of splenic or hepatic enlargement. There is reason to doubt that this represents a special form of erythremia. Parkes Weber (15), one of several clinicians who, following Osler, devoted much attention to this disorder, emphasized that hypertension is unusual in erythremia. Many have noted that cardiac hypertrophy is relatively uncommon. Peacock (16) showed that increases in blood volume or viscosity do not cause an increase in blood pressure. It has also been demonstrated that a decrease of the polycythemia in cases in which hypertension exists does not necessarily cause a fall in blood pressure.

Osler referred to the occurrence of erythromelalgia and was impressed with the association of vascular disease with erythremia, topics which have since received additional consideration (17, 18). Following Osler, important papers were published in the succeeding years by Weintraud (19), Lommel (20), Senator (21), Hirschfeld (22) and Mackey (23) and the first complete post mortem examination was reported by Weber and Watson (24). By 1912, Lucas (25) found published records of 189 cases, of which 123 can be accepted without any doubt as cases of erythremia.

One significant feature failed to impress Osler. This was the leucocytosis and the other signs of increased bone marrow activity which are so often encountered in erythremia. A leucocyte count greater than 10,000 per c mm was present in half of Osler's patients. He also did not recognize the fact that the platelets may be increased in this disease. Turk, whose proposal that the disorder be named "erythremia" was accepted by Osler, pointed out as early as 1902 that evidence of a combined increase of erythropoiesis and leukopoiesis is an important characteristic of the condition.

Studies conducted since Osler's time have yielded quantitative data supporting many of the assumptions he made but have produced remarkably little in addition (2). Thus, measurements of the blood viscosity have shown it to be 5 to 8 times the normal and the specific gravity of the blood has been found to be 1.075 to 1.080 (normal 1.055 to 1.065). The total mass of red cells has been shown to be greatly increased. It has been demonstrated that the total blood volume is

greater than normal although it is not as much increased as would be expected from the gain in red cell mass, there being, in most instances, a reduction in plasma volume

It has been shown, also, that the circulatory minute volume and the velocity of the blood flow are decreased. The skin capillaries have been found to be distended and enlargement of part or all of the capillary loops has been noted, particularly in the venous segments. All of the available vessels, in short, are open in this disease. Brown and Giffin (26) pointed out that the additional vascular space in the skin leads to a loss or impairment of the physiological heat mechanism with the result that many sensory symptoms such as intolerance to heat and cold and burning sensations develop.

Osler's conclusion that erythremia cannot be attributed to decreased destruction of the red cells found support in studies of the pigment metabolism in this disease (32). These indicated that, if anything, blood destruction is exaggerated. It was suggested later that an increased corpuscular "life span" might account for the disorder but, so far, no adequate data have been offered in support or in contradiction of this hypothesis. As already quoted, Osler was intrigued by the fact that here was a condition "in which so far as we know there is an oversupply without any corresponding demand." The cause of this increased activity of the hemopoietic system is as obscure today as it was in Osler's era. The factors which govern the physiologic regulation of hematopoiesis are still unknown and it is still a mystery why and how the blood remains "normal."

The respiratory minute volume has been found to be increased and the vital capacity reduced, the latter perhaps as the result of the intense pulmonary vascular congestion. The observation that the oxygen saturation of the arterial blood is within physiological bounds has been confirmed recently (27), even in cases with volumes of packed red cells as high as 81 per cent. The hemoglobin is normal in all respects in this disorder and the oxygen unsaturation of the venous blood is within normal limits (28). The contradictory report (29) that the hemoglobin in polycythemia vera does not give up its oxygen as readily to the tissues as does normal blood has found no support in more recent studies (30). There is also nothing to confirm the suggestion (31) that there is a decrease in the pulmonary permeability to oxygen. It has

been reported that during exercise there is a significantly increased arterial oxygen unsaturation (31) but these observations await duplication

It follows that the abnormal color of the skin is due to the color the blood assumes when an abnormally large proportion of reduced hemoglobin is present. The vascular tree being filled to its greatest capacity and there being marked slowing of the blood flow, cyanosis occurs, but its intensity depends on the number and distribution of the peripheral capillaries and the subcapillary venous plexuses as well as on the thickness and amount of pigmentation of the skin.

Osler was led by his thoughts concerning "oversupply without any corresponding demand" to draw an analogy with leukemia. Consistent with this view are the cases in which a very striking leukocytosis is present and immature myeloid leukocytes are found in the circulation, those instances in which, in spite of a clinical and hematologic picture of erythremia, at autopsy the amount of leukoblastic tissue and all the pathologic findings closely resembled those characteristic of chronic myelocytic leukemia, the cases in which, after a variable duration with a course typical of erythremia, anemia developed while the leukocytosis remained high or became still greater, the cases of erythremia terminating in a classical picture of acute myeloblastic leukemia, and, finally, the cases of chronic myelocytic leukemia in which the later course and clinical picture were those of erythremia (2, 33). The Italian school of hematologists (Ferrata, Di Guglielmo) strongly support the concept that erythremia and leukemia depend on a single pathologic factor which, in erythremia, consists in a reversion on the part of the "clasmatocytoid hemohistioblast" (the primitive blood cell) to its embryonic activity so that it produces erythrocytes as well as leukocytes.

From time to time, however, attempts have been made to relate the development of erythremia to anoxia. Reference has been made already to the suggestion that the hemoglobin may be abnormal in that its oxygen combining power may be decreased. It has been proposed that the capillaries may be so dilated that the red cells cannot unload their oxygen or the tissues may be abnormal and tissue respiration may be increased. No adequate support for these hypotheses has been offered, however.

In erythremia, in contrast to secondary polycythemia, the percentage oxygen saturation of the arterial blood is normal. Consequently, it should be clear that a theory of anoxia applied to this disease requires that anoxia be produced by circumstances local to the bone marrow. Consistent with this is the report of Reznikoff, Foot and Bethea (34) who observed marked narrowing and fibrosis of the arteries and arterioles in the bone marrow in certain cases of erythremia. It remains to be shown that these vascular and hematologic changes were etiologically related and not merely coincidental. Direct measurements of the oxygen saturation and tension of samples of blood removed from the sternal marrow cavity (35) have demonstrated no significant differences between normals, anemic patients and patients with erythremia. However, the techniques used were not adequate even to demonstrate a lowered percentage oxygen saturation of the marrow blood in anemia, where an anoxic stimulus is generally believed to be the cause of increased erythropoiesis. Consequently, these negative results cannot be regarded as denying a possible role of oxygen lack as a cause of erythremia.

The writer is inclined, nevertheless, to favor the view that erythremia is similar in its pathogenesis to leukemia and is not attributable to the influence of anoxia on the bone marrow. It is clear, however, that the subject of the pathogenesis of this disorder is an open one. It would seem worthwhile to reevaluate the significance of the variety of studies which have been reported hitherto and perhaps also to explore the subject experimentally. Thus the polycythemia produced in animals by feeding cobalt might be studied and its mechanism investigated. It might be of value to determine whether it is really true, as everyone assumes, that anoxia stimulates erythropoiesis by a direct effect on the bone marrow (36). It seems possible that the effect of rarefied atmospheres in stimulating erythropoiesis is mediated through some other organ. Discovery of such a hypothetical mechanism might prove of value in disclosing the factors governing hemopoiesis.

Although there has been so little progress since Osler's time in gaining an understanding of the pathogenesis of erythremia, advances in the management of the disorder, fortunately, have not been held to this slow pace. In addition to venesection, a procedure initiated in Osler's time and utilized effectively to this day, a variety of methods of treat-

ment have been devised. These include impairment of blood regeneration following venesection by feeding an iron-poor diet, destruction of the blood by the administration of phenylhydrazine, and destruction of hemopoietic tissue by roentgen irradiation, especially "spray" therapy (37), by the administration of nitrogen mustard (38) or by injecting radioactive phosphorus ( $P^{32}$ ). Of these procedures, the last has achieved the most favored position.

Radioactive phosphorus passes to tissues which have a high phosphorus content and which metabolize phosphorus rapidly. Since the half-life of  $P^{32}$  is 14.3 days, steady irradiation of tissue takes place for several weeks. The concentration of radioactive phosphorus in bone makes it particularly valuable in the management of hemopoietic disorders and, of these, the most impressive beneficial effects have been observed in erythremia. Lawrence (39), in a series of 115 cases, reports satisfactory clinical and hematological remissions lasting a number of years. Many of his patients received only one course of therapy (usually 2 injections of 3-6 mc), some of them not needing retreatment after 4, 5, 6, 7 and 8 years. The average duration of life in his cases has been approximately 17 years, a life expectancy similar to that of patients with diabetes mellitus treated with insulin and of patients with pernicious anemia treated with liver extract. Hall (40), in a series of 124 cases, noted a reduction of hemorrhagic manifestations from 33 to 1.6 per cent, while the incidence of thrombosis decreased to 2.4 per cent from 27.4 per cent. In each series, however, acute leukemia was observed 5 times, an incidence which is higher than had been noted prior to the use of this therapeutic agent (41).

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# A CONSIDERATION OF THE BANTI SYNDROME

"Even in well known affections advances are made from time to time that render necessary revision of our accumulated knowledge, a readjustment of old positions, a removal even of the old landmarks "

SIR WILLIAM OSLER, 1895

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The purpose of this paper is to cite a few of the publications pertinent to the evolution of the concept of the Banti syndrome which have appeared since Osler's first reports (1-7) on the subject. Although originally considered a separate disease entity, the term is now applied to conditions of various etiologies having in common anemia, leukopenia, occasionally thrombocytopenia, splenomegaly and evidence of hepatic disease or altered portal circulation.

## I

*Incidence* Although relatively uncommon (8-10) such cases are the subject of numerous papers. The incidence has been reported equal in the two sexes (11-14). Occupation and heredity have been stated as playing no role (4, 11, 12). Cases indicating a possible familial incidence have been described (4, 9, 15) though Banti would not accept one such series (16) as an example of his entity. The condition seems to be more common in white than colored patients (17). The younger age group has predominated in several series (4, 9, 11, 17, 18, 19). No unusual geographic distribution has been noted (18, 20).

## II

*Symptoms and signs* The following paragraphs present in summary Banti's original description of his clinical observations. The sequence of events he recorded is rarely seen. The clinical symptoms and signs are simply those of anemia, splenomegaly, unusual bleeding (most frequently from esophageal varices), altered portal blood flow and liver disease, symptoms and signs which may be seen in a variety of diseases.

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# EDINBURGH MEDICAL JOURNAL.



## ORIGINAL COMMUNICATIONS.

### CHRONIC SPLENIC ENLARGEMENT WITH RECURRING GASTRO-INTESTINAL HÆMORRHAGES

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EXCLUDING the enlarged spleen of leukaemia, chronic malaria, cirrhosis of the liver, heart disease, and rickets, the cases of so-called primitive enlargement of the organ fall into two groups.

First, a series in which the spleen is enlarged without causing any symptoms, other than those due to mechanical pressure. In the past few years I have seen four patients, all women, apparently in perfect health, who complained only of a feeling of pressure in the abdomen, in all of whom the spleen was much enlarged. In two cases in which the organ was freely movable and caused a great deal of discomfort, my colleague Halsted opened the abdomen and successfully packed the spleen in position with gauze, an operation much less serious than splenectomy and very efficacious. Both these patients have been seen more than two years subsequent to the operation, and have remained quite well. In a third case, a girl was sent into the gynaecological department, supposed to have an ovarian tumour. She was robust and strong, with good colour, and had been hard at work. She subsequently had a twist of the ligaments and sphacelus of the spleen, with enormous enlargement, adhesion to the abdominal wall, redness, and inflammation. The organ was freely incised by Halsted, and an enormous quantity of necrotic spleen tissue removed, the patient made a good recovery. This condition is, I think, more common than we suspect. The spleen is, as a rule, only moderately enlarged. In some cases there has been a history of past malaria, but in a majority the condition is one of, so far as we can tell, primary enlargement. It must not be forgotten that only a blood examination can determine whether or not such patients have leukaemia, since in this disease, as is well

Banti (19, 20, 21) described three clinical phases. Phase I was termed the preascitic or anemic stage. He believed that the enlargement of the spleen always preceded other signs and symptoms. Usually the onset was insidious with increasing pallor, easy fatigability, palpitation and dyspnea, occasionally there was ankle edema if anemia were severe. Epistaxes were frequent. Sometimes pain or a feeling of weight in the left hypochondrium led to the discovery of a large spleen and anemia. Characteristically the spleen was not tender or painful (unless perisplenitis occurred). The splenic enlargement gradually increased until, infrequently, the organ reached the pelvic brim. Banti thought brief episodes of fever and chills were not uncommon in this period. Although he denied evidence of hepatic disease during this stage, his only clinical criteria were inability to detect hepatic enlargement, jaundice, evidence of portal collateral circulation or increased urobilin in the urine. In his opinion, this stage of the disease lasted usually from 1 to 4 years or longer. Phase II was ushered in by gradual enlargement of the liver until it extended two to three fingerbreadths below the costal margin. The anemia increased. No ascites was apparent. The spleen did not enlarge further. Jaundice was observed only occasionally, and was thought due to liver damage and not to increased blood destruction. The urine was found to contain urobilin and sometimes bilirubin. Banti thought this period lasted generally only a few months. Phase III was characterized by ascites, associated with a decrease in the size of the liver. Jaundice appeared. The spleen remained unchanged in size. Anemia increased in severity. Gastrointestinal hemorrhages were common. Urinary bile pigments increased in amounts. Death in these cases was ascribed to either gastrointestinal hemorrhage or hepatic insufficiency. Clinically Banti considered the third phase indistinguishable from atrophic (alcoholic) cirrhosis. He differentiated these cases from what he called splenic anemia because they progressed from a state of splenomegaly with anemia to one characterized by marked liver damage with cirrhosis, jaundice, collateral portal circulation and gastrointestinal hemorrhages.

Criticisms of Banti's original clinical description have been frequent. Eppinger (22) emphasized the extreme rarity of such a clinical history. In his wide experience he had seen only one possible case. McNee (23) stated that he never saw a case agreeing really closely with the sequence of events originally described. Furthermore, clinical states similar to the last or third phase as described by Banti have been observed in association with Laennec's cirrhosis (24), syphilis (7, 24, 26) "healed acute yellow atrophy" (27), schistosomiasis (28), hemochromatosis (27), post traumatic abdominal lesions (10, 12), aneurysms of the splenic artery (29, 30), cavernous transformations of the splenic vein (31), developmental anomalies of the portal system (30), thrombophlebitis of the portal and splenic veins (32), thrombosis of the splenic and portal system (12, 33, 34), persistence of the umbilical vein (35),

polycythemia (36), extension of the thrombosis of the umbilical vein at birth to involve the portal system (32, 37), obstruction of flow by periportal adhesions or extrinsic abdominal masses (34, 38) and infectious hepatitis (39). To expect one characteristic sequence of clinical events does not therefore seem logical. Rousselot (12) drew the conclusion that the clinical picture represents a heterogeneous group of cases better called Banti's syndrome than Banti's disease.

Recurrent hematemesis or melena is not uncommon, occurring in about one-half of the cases diagnosed as Banti's syndrome (40, 41). Sometimes such gastrointestinal hemorrhage occurs without previous appearance of any other signs or symptoms (13). Epistaxes of severe degree in these cases are not infrequent (11, 19) and may be the only evidence of unusual bleeding (12). There seems to be no direct correlation between epistaxes and platelet count (12). Purpura occurs occasionally (4, 25, 42), may be recurrent (2) and associated with bleeding gums and ecchymoses (40).

Commonly, due to the enlarged spleen, the initial complaint concerns a mass in the abdomen (11). Usually, the spleen is quite firm and is not tender. Its size has been reported as varying inversely with the age of the patient (43). Splenic enlargement may decrease transiently after a severe gastrointestinal hemorrhage (12). Occasionally a friction rub or a thrill may be palpable over the spleen and a bruit may be heard (44). Transient, sometimes severe, abdominal pain occurs (11) and may be due to infarctions with perisplenitis or a twisted splenic pedicle. When hepatomegaly is present the enlargement is not great unless schistosomiasis is the etiological factor.

Although ascites occur in about one-third of the cases (14) enlargement of the peripheral abdominal vessels is rarely noted (11, 20). Osler (2) reported that one patient (Case IX) had three separate severe episodes of ascites though at autopsy there was no cirrhosis of the liver. Rolleston (44) observed ascites in the absence of cirrhosis. Ravenna (45) considering the possible etiology of the syndrome stressed the rapid disappearance and recurrence of ascites in certain cases.

In the presence of splenomegaly and ascites anorexia is common, nausea and vomiting and diarrhea are infrequent (12). Cardiac symptoms seldom appear unless the anemia is severe (12). Dyspnea, palpitation and precordial distress may then develop in that order. De-

pendent pitting edema of mild degree is common. Urinary symptoms are extremely rare although hematuria has been observed (2). Peripheral lymph-nodes are usually not enlarged. Weight loss in one series of twenty-two patients averaged only about five pounds (11). Osler (2) and Giffin (46a) commented on melanoderma in several of their cases. A skin biopsy of one such patient failed to reveal any iron-containing pigment. Such skin pigmentation may have resulted from extensive use of Fowler's solution which was then in therapeutic vogue. Spider hemangiomas (5, 39) have been described.

### III

*Laboratory Data* These indicate that there is nothing characteristic about the anemia, bone marrow or peripheral blood differential count.

Usually these cases show a moderate hypochromic or normochromic normocytic anemia (14, 46). Banti stated the red blood cells generally range from 3 to 4 million per cubic millimeter. In three large series of cases (4, 11, 17) the red blood cell count averaged 3,490,000 per cubic millimeter and in two of the series the hemoglobin averaged 54 per cent with a color index of 0.62. The majority of Larrabee's forty-seven cases (27) showed a microcytic anemia but macrocytic anemia has been observed (14, 47, 48). Wright (48) reported a case displaying a transition from a hypochromic microcytic anemia to a macrocytic anemia. Poikilocytosis and anisocytosis are reported frequently and polychromatophilia occasionally. The reticulocyte count may be normal although reticulocytosis does occur (46, 49) particularly after hemorrhage. Although Banti emphasized the absence of nucleated red blood cells in the peripheral blood (19) these have been reported frequently with this diagnosis (11).

Banti observed leucopenia and granulocytopenia with occasional absolute decrease in lymphocytes. The monocytes may show a relative or even absolute increase (20). Blast forms of the white blood cells in the peripheral blood have not been described in any of several large series of cases (4, 11, 12, 17), although "myeloid immaturity" has been reported (50). The leucocyte count is usually below 5,000 (14) averaging 4,616 for the patients in four large series (3, 9, 11, 20). The white blood cell count may be as low as 320 per cubic millimeter (51). Following sudden hemorrhage the count may exceed 12,000.

per cubic millimeter (2) and after splenectomy the white blood cell count may rise to very high levels and remain elevated for the remainder of the patient's life (12)

The platelet count is frequently decreased (42, 46, 52) Occasionally, however, it may be normal or relatively high (31, 53) Cases of Banti's syndrome with no decrease of platelet count have been called "thrombocythemic" by Rosenthal (25) He reported that in such cases splenectomy was followed by a very rapid rise and permanent elevation of the platelet count In two of his cases the count remained over 1 million and in another it ranged from 600,000 to 800,000 for two and one-half years following splenectomy Platelets two to four times normal size may be seen occasionally in the peripheral blood (25) The significance of the apparent lack of correlation between platelet counts and bleeding tendencies in such cases cannot be evaluated in the absence of simultaneous data on prothrombin times (25, 42) However, Morlock and Hall (42) have reported a case of hepatosplenomegaly associated with bleeding tendencies with a platelet count of 56,000 to 68,000 and a normal prothrombin time Tocantins (54) concluded the bleeding tendency was due to both thrombocytopenia and hypoprothrombinemia Even in the absence of the Banti syndrome liver disease may be associated with thrombocytopenia (42, 55-60) Increased capillary fragility has been observed (25)

Coagulation time and clot retraction, when studied, have been found usually within normal range (12, 46) However, Rosenthal (25) observed impaired clot retraction in a few cases and its actual absence in one Osmotic fragility of the red blood cells is frequently normal (12, 61, 62) However, Boulton (49) observed increased osmotic fragility, though at the time the reticulocyte count was 30 per cent McMichael (51) carried out osmotic fragility studies in fifteen cases In six the fragility was normal, in seven it was decreased and in two it was increased, with hemolysis beginning at 0.55 per cent to 0.6 per cent saline

Banti (20) reported in his autopsied cases an increase of the amount of "red" marrow with many nucleated red blood cells Dock and Warthin (63) described an increased number of normoblasts in the marrow, numerous phagocytes containing blood pigment and an occasional giant cell An increase in erythropoiesis has been observed by others

(2, 64) Segerdahl (65) has reported marrow from one case as hyperplastic with immature erythrocytic and normal myelocytic cells Mendell et al (66) reported there were no characteristic marrow changes in the three cases they studied Hypoplasia of the marrow also has been described as characteristic (13) Limarzi et al (50) reported in detail on the bone marrow and blood in several cases of Banti's syndrome In the earliest stages the marrow displayed a myelocytic hyperplasia while in the peripheral blood there was a moderate anemia and leukopenia Later in the disease the marrow showed maturation arrest of the myelocytic and megakaryocytic tissue with leukopenia, neutropenia and thrombocytopenia and myelocytic immaturity in the peripheral blood In the last stages of the condition, when cirrhosis of the liver was severe, they observed marked immaturity of both the erythrocytic and myelocytic series

#### IV

*Pathogenesis* In the following paragraphs Banti's opinion of the pathogenesis of this syndrome is presented for its historical interest The evidence for considering increased portal and splenic vein pressure as the "common denominator" is outlined, as well as the various possible causes for such hypertension that have been suggested It is pointed out that if such portal or splenic vein hypertension occurs in these cases its relationship to the anemia, leukopenia and thrombocytopenia remains unexplained Although blood loss from varices and impaired hepatic function have been considered important factors it is possible the spleen itself may have an etiological role A variety of evidence is considered suggesting that the spleen may destroy cellular elements of the blood excessively, or may inhibit hematopoiesis Several possible objections to these interpretations in this syndrome are then presented No conclusion is warranted with the present evidence about the exact role of the spleen in this syndrome

Originally Banti (21) considered the syndrome the result of a "poison" produced in the spleen In his last long article (20) on the subject he stated that some "infectious" agent probably reached the spleen through the arterial blood supply and caused primarily perarteriolar lesions (which, when extensive, he called 'fibroadenie') He maintained his original opinion that the splenic lesions and enlargement were not secondary to any liver disease and listed five reasons for differenti-



✓ ating his entity from that of Laennec's cirrhosis (21) His reasons for considering liver disease as absent in the early stage have been presented and are not adequate in the light of present day knowledge His main premise for considering the spleen an etiological factor was his impression that splenectomy was curative

Banti listed three observations from which he concluded the anemia was not due to increased blood destruction (1) Microscopic examination of the splenic juice rarely showed any phagocytosis of red blood cells (2) There was no increased pigmentation in the organs at autopsy (3) There was no increase in number of nucleated red blood cells in the splenic pulp or extramedullary erythropoiesis

As early as 1904 Dock and Warthin (63), following observations on two cases of portal obstruction, expressed the opinion that the splenomegaly of Banti's syndrome was the result of obstruction of the portal and splenic veins Wallgren (37), in a discussion of a variety of extrahepatic lesions associated with portal vein obstruction, pointed out the similarities between such cases and those diagnosed as Banti's syndrome Several others (27, 38) described the Banti syndrome associated with various abdominal lesions obstructing the venous outflow of the spleen Rousselot (12) and Thompson (34), after reviewing a large number of cases diagnosed as Banti's syndrome, came to the conclusion that venous obstruction was the "common denominator" Splenic vein pressures have been reported markedly elevated (67) in several cases of Banti's syndrome In fourteen cases the splenic vein pressures ranged from 225 to over 500 millimeters of saline However, twenty-two out of fifty-five carefully studied cases failed to show any obstructive factor (68)

Other possible causes of increased splenic vein pressure besides portal and splenic thrombosis have been described Herrick (69) perfused normal and cirrhotic human livers through the hepatic artery under controlled pressures and measured the amount of fluid flowing from the portal vein He concluded there was freer communication between the hepatic artery and portal vein in cirrhotic than in normal livers and that this communication probably was an important factor by which the arterial pressure could increase the portal pressure in the presence of cirrhosis of the liver McIndoe (70) was unable to confirm Herrick's studies However, Dock (71), on the basis of perfusion experiments, concluded that in cirrhosis there was damage to the intrahepatic arteriportal anastomoses and that in some cases the arterial pressure probably contributed significantly to the portal vein pressure

Johnston (43) made camera lucida drawings of the terminal portal sheaths and the immediately accompanying hepatic arterioles in ten cases dying of acute infection and four previously diagnosed as Banti's syndrome. The average ratio in each case of hepatic artery lumen (Ha) to portal vein lumen (Pv) circumference was calculated (Ha-Pv) and found decreased in the Banti syndrome cases. There appeared to be no relationship between the changes in Ha-Pv ratio and fibrosis of the liver. Johnston concluded that constriction or reduction of the size of the portal venules was not simply the result of compression by contracting scar tissue since as much or more reduction was noted in livers with little fibrosis as in those most extensively sclerosed. The size of the spleen was inversely proportional to the size of available vascular bed. McMichael (72, 73) has suggested two other possible causes of increased portal pressure: (1) vasodilatation of the hepatic artery and arterioles and (2) vasospasm of the portal vein. Burton-Opitz (74) demonstrated sudden marked rises in portal pressure following the injection of small amounts of adrenalin into the portal vein of dogs. The slight rises in arterial pressure were not proportional and were more delayed. He interpreted this observation as supporting the idea that intrahepatic portal radicles have a constricting capacity. This conclusion has been substantiated by other experiments (75, 76). However, Popper (77) has shown that the walls of the portal vein radicles of man have much less smooth muscle than those of dogs.

Even if one assumes that elevated portal or splenic vein pressure is the "common denominator" in these cases, the actual mechanism of influence on the hematological tissues must be explained. Sturgis (14) considers the liver impairment and the blood loss from varices adequate reason for the anemia.

It has been assumed the elevated splenic vein pressures are associated with an increased amount of hemostasis in the spleen. Numerous experiments suggest that this may damage the red blood cell. Ham and Castle (78, 79, 80) have repeatedly emphasized the role of stasis in various anemias. Bolt and Heeres (81) demonstrated that red blood cells in the splenic vein showed decreased resistance to hypotonic saline. Heilmeyer (82) found that red blood cells become spheroidal during passage through the spleen. Bergenheim and Fah-

raeus (83) emphasized the effects of stasis *in vitro* and suggested the same effects might occur in the spleen. They found that incubation of defibrinated blood at 37°C without stirring caused spherocytosis. From incubated serum Bergenheim and Fahraeus isolated a water-soluble, ether-insoluble, phosphorus-containing hemolysin. They assumed from further work with cobra venom containing lecithinase and enzyme inhibitors that "lysolecithin" was formed during sterile incubation of serum or citrated plasma. Two possibilities were suggested to explain the lack of spherocytosis occurring in incubated blood if it were continually agitated: either lysolecithin was not formed (presumably due to inhibition of serum lecithinase) or if formed, was immediately inactivated as a result of the circulation and contact of the serum or plasma with the red blood cells. Fahraeus (84) later quoted some of Knisely's experiments (85) to support his contention that such effects could occur *in vivo* in the spleen. Knisely studied the splenic vascular circulation of mice, rats and cats by direct visualization. He found the splenic sinuses capable of storing and concentrating red blood cells. Storage and concentration of red blood cells in the spleen of animals had been demonstrated previously by several other workers (86, 87, 88). Splenic storage of red blood cells may not be always intrasinusoidal (88a, 88b). Watson and Paine (89) were the first to show an increase in hematocrit in the splenic vein in man after the administration of adrenalin, thereby demonstrating a storage of red blood cells in the spleen. Among the nine cases they studied was one diagnosed as cirrhosis of the liver with congestive splenomegaly. Before injection of adrenalin the hemoglobin, hematocrit, red blood cell count and osmotic fragility of the red blood cells in blood removed simultaneously from the splenic artery and vein were practically identical. However, after the injection of adrenalin into the splenic artery, the splenic vein hemoglobin, hematocrit and red blood cell count rose dramatically and the osmotic fragility of the splenic vein red blood cells increased. Of even greater interest was the observation that the mean corpuscular hemoglobin concentration in the red blood cells of the splenic vein decreased. Watson and Paine suggested that during hemoconcentration and stasis in the spleen there was actual intracellular degradation of hemoglobin. A wide variety of other evidence has been interpreted as suggesting that the spleen is capable of alter-

ing and presumably damaging red blood cells passing through or stored within it. Rich and Rienhoff (90) observed a higher serum bilirubin content in the splenic vein than in the splenic artery, indicating red blood cell breakdown within the spleen. The lysolecithin content of splenic blood has been found higher than that in peripheral blood (47). Gripwall (91) isolated from the spleen a hemolytic substance similar in chemical properties to lysolecithin. Wasastjerna (92) reported that a heterologous anti-red blood cell serum with agglutinating and hemolyzing activity was more effective in normal than in splenectomized animals. Granick (93) observed an increase in non-hematin iron in red blood cells within the splenic tissue of horses.

However, the importance of the role of passive congestion in the spleen as an etiological factor in this syndrome has been questioned since the time of Banti himself. Dawson (94) reported that of one hundred and seven cases of thrombosis of the portal and splenic veins found at autopsy only thirty had splenomegaly, and of these only eighteen had had the clinical picture of "splenic anaemia". He concluded that such thrombosis of the portal and splenic system is not frequently associated with this syndrome. Attempts to produce it experimentally by interfering with the portal circulation have failed (72). Warthin (32) ligated the splenic vein but atrophy of the spleen resulted. Although Jager (95) produced slight splenic enlargement by intermittent splenic and collateral vein obstruction in dogs, the spleen never exceeded the dimensions attained in the initial congestion. Rousselot and Thompson (96) produced cirrhosis of the liver, splenic vein hypertension and splenic enlargement in dogs after repeated injections of silicon dioxide (silica) into the splenic vein. No hematological studies were reported. Burton-Opitz (74) stated in one of his numerous papers on experimental studies of the portal circulation that increases in portal vein pressure were not necessarily associated with any decrease in portal flow. McMichael (72) concluded from his observations and those of Jager (95) that enlargement of the spleen in excess of 500 grams in a human adult must involve some factor other than mere passive congestion. McMichael (72), Menon (97) and Cameron and deSaram (98) have insisted that hyperplasia of the pulp cells and endothelium indicates that some factor in addition to passive splenic congestion is of etiological importance.

Ravenna (45) concluded on 'the basis of six premises\* that portal obstruction and splenic passive congestion were not necessary factors in this syndrome, but that instead, splenic arteriolar dilatation and active hyperemia occurred. As early as 1902, Barr (100) suggested that the splenic changes in Banti's syndrome were due to splanchnic vasomotor paresis. He reported marked alteration of the semilunar ganglia of his three cases: lymphocytic infiltration with fatty and pigment "degeneration" of the ganglia cells. If there were sufficient increase in arteriovenous communications and blood flow in the spleen to cause marked congestion and increased portal pressure the blood volume might increase. Blood volume determinations apparently have not been done with this possibility in mind. In support of a splenic arteriolar lesion are the observations reported by Bouchard during his discussion of one of Osler's papers (5) on splenic anemia. He had noted in some patients with cirrhosis of the liver and splenomegaly the simultaneous occurrence of a murmur over the spleen, clubbing of the fingers and numerous spider hemangiomas. He reported that the hemangiomas and splenic enlargement occasionally receded together.

Doan (101) has stated that, although the anemia observed in the Banti syndrome is probably due partially to blood loss from esophageal and gastric varices, increased sequestration and destruction of white blood cells and platelets in the spleen may account for the leucopenia and thrombocytopenia. He has been impressed by the relative lack of leucocytosis following severe gastrointestinal hemorrhage.

\* (1) Ravenna reported twelve cases of splenic and portal vein occlusion without splenomegaly. (2) Some cases diagnosed as Banti's syndrome have not shown any obstruction in the portal system even though the splenic vein pressure was high. (3) Frequently lesions have been found about the splenic arterioles suggesting these may have been the primary site of change causing splenomegaly and elevated splenic and portal vein pressures. (4) By denervation of the spleen (99), splenomegaly has been produced in dogs, indicating again a splenic vascular change as a potential cause of such enlargement. (5) Transient recurrent episodes of ascites with sudden onset and rapid disappearance have been reported, suggesting a reversible process as the cause. (6) Lastly, Ravenna pointed out that dilatation of portal vessels may occur on the hepatic side of a localized obstruction in the splenic or portal vein indicating an increased and not such a decreased blood flow as one might logically assume to occur with thrombosis. He concluded that in such cases the thrombosis may have been secondary and not primary.

in such patients Doan and Wright (102) stated that increased phagocytosis by splenic macrophages can be incriminated in all cases of "hypersplenism" Doan (103-105) and Wiseman and Doan (106) reported that the reticuloendothelial cells of the spleen may occasionally phagocytize red blood cells, platelets and granulocytes excessively in a selective or indiscriminate manner Menon (97) observed increased erythrophagocytosis in the spleens of rabbits and rats previously injected with manganese chloride and senecionine In 1911 Lintwarew (107) published some amazing pictures of extreme phagocytosis of red blood cells in both spleen and liver However, in evaluating the significance of intracellular matter in macrophages studied in supravital stained preparations of splenic tissue it must be remembered that a certain amount of phagocytosis may occur after death of the patient or surgical removal of the spleen Rich (108) has shown that ingestion of red blood cells by macrophages can occur in vitro Therefore, phagocytosis in such preparations may not necessarily indicate excessive phagocytosis in vivo Von Haam and Awny (109), after studies on fixed smears stained by Giemsa's method and on smears stained by supravital techniques from cases of "hypersplenism", concluded there was some increase in number of endothelial cells and perhaps a slight increase in phagocytosis but the latter activity could not cause any great destruction of red blood cells, platelets or white blood cells

Some observers have postulated a hypothetical effect of the spleen upon the bone marrow, presumably due to a splenic hormone (110) Shousboe (111) after studying a case of Banti's syndrome, concluded that the spleen controlled cell emission from the bone marrow Before splenectomy his patient showed only occasional nucleated red blood cells in the periphery but, after splenectomy, and at comparable hemoglobin levels, 34 per cent of the nucleated cells in the peripheral blood were of the red blood cell series Shousboe's conclusions from his bone marrow studies are, however, open to question because two of the sternal aspirations followed very severe gastrointestinal bleeding Limarzi et al (50), in their studies of the bone marrow in Banti's syndrome, found that, although a marked decrease in hyperplasia occurred after splenectomy, the anemia improved, they concluded that a maturation-inhibitory substance had been produced by the enlarged

spleen Pearce, Krumbhaar and Musser (112) reported anemia of three to four months duration following splenectomy in dogs, and assumed that the transient anemia was due to a loss of some splenic factor which ordinarily stimulated erythropoiesis in the bone marrow. They felt they could shorten the period of anemia by the administration of splenic extracts. Leake and Bacon (113) followed the red blood cell counts over a period of several days following the administration of bone marrow and splenic extracts intravenously to rabbits and orally to dogs. They concluded that their extracts contained thermostable, water-soluble erythropoietic agents, which acted first by increasing the rate of production or delivery of existing erythrogenic centers and later caused an extension of the functioning red marrow. However, the hemoglobin levels did not change significantly in their studies. Stradomsky (114) cited the appearance of Howell-Jolly bodies in circulating red blood cells following splenectomy as evidence that the spleen affected maturation. Although Dameshek and Miller (115) suggested a possible hormonal mechanism inhibiting megakaryocytes from producing and releasing platelets in some cases of thrombocytopenic purpura, they found that in cases of splenic vein thrombosis and cirrhosis the megakaryocytes of the bone marrow were increased in number and platelet production seemed normal.

It should be pointed out that, if the spleen forms some factor inhibiting bone marrow activity, which, it has been suggested, may be formed in excess in cases of "hypersplenism", it seems odd that the spleen so frequently becomes one of the first sites of hematopoietic metaplasia. Furthermore, if the spleen forms some factor stimulating maturation, and inhibiting release, of very young hematopoietic cells it is rather contradictory that so many blast forms occur frequently in the peripheral blood in some cases in which the spleen has become one of the main sites of blood formation (116).

The suggestion (117) of streptothrix being demonstrable in the spleen and, therefore, being the cause of the Banti syndrome is of historical interest only, such histological appearances were not due to fungus (72). Nor are the experimental studies (118) on the possible relationship of infection to the syndrome admissible in light of present day knowledge.

## V

*Pathology of the spleen* According to the following descriptions the splenomegaly is frequently associated with microscopic evidence of recent or old hemorrhage, most marked around the penicillar arterioles which may be occluded. There is commonly an increase of connective tissue in the splenic pulp and around the malpighian follicles. The cellular elements of the pulp may or may not increase. The splenic vessels and portal vein may be dilated, tortuous and sclerotic and the veins may even be thrombosed. Whether such a histological picture is associated with any unusual splenic function is unknown.

Banti (19-21) described lesions in his cases in the spleen and liver, and frequently in the splenic and portal veins. He stated that, typically, the spleen was enlarged, smooth and of normal shape, the capsule was often unchanged or occasionally thickened, the weight was seldom below 1,000 grams but might be as large as 3 kilograms. On section the parenchyma appeared firm and red and the malpighian bodies were usually easily seen. Banti applied the term "fibroadenoma" to the fibrosis observed around the malpighian follicles and thought that the fibrosis resulted from localized adenitis or inflammation of each follicle, extending into the pulp and follicle as the condition progressed until occasionally in the last stage some follicles became only sclerotic nodules. As the fibrosis of the splenic reticulum increased the splenic sinuses became smaller. During the second clinical phase Banti believed that intrahepatic branches of the portal vein became thickened and sclerosed. Cirrhosis of the liver was observed in the third and last phase of the disease and was considered by Banti as indistinguishable from the portal cirrhosis of Laennec. He did not observe siderosis in either the spleen or liver. Banti reported patches of "chronic sclerotic endophlebitis" in the splenic, portal and mesenteric veins, but, he did not describe complete thrombosis of the splenic or portal veins in any of his cases (23). As the most extensive lesions were usually seen in the perifollicular area and were occasionally associated with hyaline changes of the penicillar arterioles which supply the area, Banti assumed a lesion of the arteriole to be primary.

After studying some of the splenic sections from Banti's original cases Durr (119) came to the conclusion that the changes described were indistinguishable from those seen in cases of hepatic cirrhosis.

MacCallum (120) described the appearance of the spleen from three patients of Osler's series (3). A few non-calcareous thrombi were found in the branches of the splenic veins of one case though the splenic vein itself appeared normal. Microscopically there was a great



increase in connective tissue in the pulp area. The malpighian follicles were less numerous than in a normal spleen but MacCallum suggested that the apparent diminution in number was due to the enormous increase in size of the red splenic pulp, consisting almost entirely of matted thick-walled venules. Many of the follicles were normal in appearance though some were very small, no sclerotic process as described by Banti was observed about them. Rounded mononuclear cells closely resembling plasma cells were abundant, eosinophils were numerous and polymorphonuclear cells and multinucleated giant cells were seen occasionally. MacCallum observed no extravasation of blood with hemosiderin formation and no marked widening of the venules. He did not think such splenic enlargement and induration simply followed chronic passive congestion. Menon (97), Cameron and deSaram (98) and Von Haam and Awny (109) have commented on the reticuloendothelial hyperplasia in the splenic pulp in cases of the Banti syndrome and have concluded that such a histological feature is not seen in simple chronic passive congestion of the spleen. Chaney (11) reported the average weight of the spleen in sixty-nine cases was 950 grams with extremes of 120 and 2290 grams. He observed splenic infarcts in five cases studied. The average diameter of the malpighian follicles was less than normal being 0.35 millimeters. Klemperer (121) stated that the size of malpighian follicles varies inversely with the age of the patient.

McMichael (72), after the pathological study of forty-five selected cases of splenomegaly, concluded they could be divided into two groups: (1) one in which cirrhosis of the liver was accompanied by splenomegaly and (2) one in which, although obvious cirrhosis of the liver was absent, the enlarged spleen showed pathological features identical with those in the first group. He described a variety of splenic lesions all of which he concluded derived from two types of changes: (1) hemorrhage about the penicillar arterioles which supply the perifollicular area and, (2) proliferation of reticuloendothelial cells. Sites of old hemorrhages called "siderotic nodules" were often visible grossly as rusty yellowish spots on the cut surface of the spleen and showed microscopically a small, central, frequently hyalinized, artery surrounded by a zone of connective tissue and masses of golden-yellow iron-containing pigment. Such lesions have been described by others and called "tobacco nodes of Gamma-Gandy" (121).

Moschowitz (122), assuming the splenomegaly of the Banti syndrome was always due to portal hypertension, listed the histological changes observed in the spleen at various stages following the onset of such hypertension. At first, hemorrhage about the malpighian follicles occurred and the sinuses were invisible because of engorgement of the pulp with red blood cells. Later dilated venous sinuses were observed which subsequently became smaller as proliferation of the cells in the intersinus pulp or Bilroth cord areas occurred and fibrosis appeared. The walls of the sinuses became progressively thicker and the pulp cells assumed more and more the appearance of fibroblasts. The fibrosis encroached on the follicles until they diminished markedly in size. Although the sinuses remained small they occasionally were so prominent in number that the sections resembled an angioma. Foci of hematopoieses were observed. Klemperer (121) has stated myeloid metaplasia is occasionally seen in the spleen in cases of splenic or portal vein thrombosis.

The possibility should be considered that the splenic lesion in some cases of so-called Banti syndrome may be comparable to the spider hemangiomata seen in the skin of some of the cases. However, increased splenic flow alone may not cause this syndrome for cavernous hemangiomata of the spleen, seen usually in adults before the age of 45 years, need not be associated with the symptoms of the syndrome (123) and the splenic tissue surrounding the hemangioma may be quite normal.

## VI

*Clinical course and therapy* The clinical course depends usually on the severity of gastrointestinal bleeding and the degree of liver damage. Statistically splenectomy appears the most effective treatment, though it is not always successful. The younger the patient and the less the degree of liver damage the more likely splenectomy will be beneficial.

Generally the clinical course occupies several years. Various signs and symptoms and laboratory data have been listed as indication for or against splenectomy. Hanrahan (13) concluded that, with evidence of an appreciable degree of liver damage, the late prognosis was rarely improved by splenectomy and this slight chance had to be weighed against a high immediate postoperative mortality. However, if there

were evidence of only slight hepatic damage, removal of the spleen arrested or delayed the cirrhotic process. In his experience, macrocytosis indicated a poor prognosis with or without operation, but the more marked the leukopenia and the lower the color index the more likely would splenectomy be beneficial. Chaney (11) reported twelve postoperative deaths in a series of sixty-nine and concluded cirrhosis of the liver and ascites increased the operative mortality. Of the twenty-one cases of splenectomy studied by Ehason and Johnson (124) eight died, three from "liver insufficiency" and three from gastric hemorrhage. They concluded splenectomy in Banti's syndrome had best be done in an early stage and was indicated later only if the size of the spleen made the patient's life miserable.

Rosenthal (25) reported that in those cases with normal or relatively high platelet counts thromboses following splenectomy were frequent and occasionally fatal. In such cases the platelet counts rose postoperatively and remained at very high levels for several years. In the thrombocytopenic group, however, the platelet counts began to fall in the third postoperative week and remained at normal or only slightly increased levels. Rosenthal concluded that a relatively normal platelet count was a definite contraindication to splenectomy. Klemperer (31), Evans (125) and Moore (53) have also emphasized the dangers of postoperative thrombosis in such cases. However, Whipple (24) and others (12, 126) have stated that such a correlation between preoperative platelet counts and postsplenectomy thromboses has not been apparent.

Rousselot (12, 68), assuming passive congestion of the spleen was the common etiological factor, concluded the indications for splenectomy and the long term prognosis depended on the nature and severity of the obstruction to splenic flow. In the presence of decompensated liver disease splenectomy was considered contraindicated. The late postoperative results in his cases with Laennec's cirrhosis, portal vein occlusion or stenosis were extremely poor (fourteen of seventeen died) but in other forms of cirrhosis, splenic vein thrombosis, and in the group in which no obstructive factor was found the results were considered good (seven of forty-two died). More recently Elliott (126a) also has reported that splenectomy is more effective in those cases where no obstructive factor is identified. In Rousselot's series of

twenty-one deaths, eight died within six months after operation and seven died from one to two years after operation, the longest interval between operation and death being over thirteen years. Postoperative hematemesis occurred in no case that had not had such a sign before operation. The two leading causes of death were hematemesis and hepatic insufficiency. Of eleven cases having hematemesis after splenectomy seven died from exsanguination. In three series of cases undergoing splenectomy the operative mortality ranged from 6 to 11.7 per cent (33, 41, 127, 128). Mayo (33, 127) stated that only a small percentage of patients who have had hemorrhages before splenectomy have them after operation. Three of his cases developed thromboses of the superior mesenteric and portal veins postoperatively superimposed on a recanalized splenic vein thrombosis. Davis (129) has also reported postsplenectomy portal vein thrombosis.

Pemberton (41) reviewed one hundred and sixty-seven cases of splenic anemia and Banti's syndrome which were subjected to splenectomy. Of the one hundred and fifty-one patients surviving the immediate effects of the operation (operative mortality of 9.6 per cent) eighty were living at the time of the followup, three of them eighteen years after operation. Fifteen were still living ten to fifteen years after operation while ten of the sixty-eight patients surviving the operation but dying later lived for more than nine years. Although many of the causes of death were not attributable to the disease itself more than a third were directly attributable to hemorrhage. The operative mortality of those younger than forty years was only half that of the patients over forty years of age. Pemberton concluded that operation was of great benefit in many instances even in the late stages of the disease and that increased hepatic regeneration occurred following splenectomy. There seems to be general agreement that if splenectomy is of value its effect is more marked if performed early in the clinical course and before there is prominent evidence of liver disease (13, 17, 33, 127, 94, 24, 11, 124, 130). Mayo (33) stated splenectomy was the only curative treatment and Banti (20) thought splenectomy prevented progression of liver disease.

However, McMichael (51), in a study of ten cases before and on the average of two years after splenectomy found the hemoglobin concentration to have changed only from an average of 53.3 per cent to one of

64.8 per cent. Furthermore, he found in a series of thirty-seven cases of splenectomy that the mortality was 30 per cent. He also observed cases in which liver disease continued to progress after splenectomy, leading to a fatal termination. Giffin (46a) also reported one case developing clinical evidence of cirrhosis of the liver one year after splenectomy. Sturgis (14) concluded that there was no advantage from splenectomy and the idea that splenectomy averted liver disease was untenable.

Bryce (126) and Evans (125) recommended splenic artery ligation in this condition. Such ligation has been successfully done with apparent recovery from the signs and symptoms (131). However, in one patient so treated, although gradual diminution of the spleen size occurred with a concomitant increase in leukocyte level, the symptoms persisted, the clinical course with recurrent esophageal hemorrhages and progressive hepatic cirrhosis was not altered (101). Ligation of the splenic artery should be followed by transection to prevent recanalization. Coronary vein ligation and omentopexy have been used to supplement splenectomy (41). Injection of esophageal varices with sclerosing fluids after splenectomy has been suggested by Tocantins (54). Portal shunt operations have been recommended (132). Although deep x-ray and radium have been used over the spleen such treatment is said to be ineffective (101).

Medical therapy has been considered (14) as successful as splenectomy. Diets with ample amounts of proteins and including yeast or whole liver with large amounts of iron have been recommended. Davidson (133) observed marked rises in hemoglobin following oral iron therapy. In the five patients he studied the hemoglobin rose from an average of 30 per cent to one of 80 per cent. One splenectomized patient's hemoglobin remained at 45 per cent until iron was given, following which the hemoglobin rose to 94 per cent. Such observations are excellent evidence that blood loss may account to a great extent for the anemia in some cases. DeLangen (61) found impaired iron absorption in one case that improved after splenectomy.

#### CONCLUSION

✓ The Banti syndrome is characterized by anemia, leukopenia, occasionally thrombocytopenia, splenomegaly and evidence of hepatic



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# THE EFFECT OF SODIUM WITHDRAWAL UPON THE BODY WEIGHT OF NORMAL YOUNG MEN<sup>1</sup>

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## INTRODUCTION

It is now well recognized that adrenal cortical activity plays an important role in regulating the salt and water equilibrium of the body, and exerts certain effects upon blood pressure as well. Adrenal insufficiency results in the loss of sodium chloride and water, at times to the point of extreme dehydration (1), and hypotension is a frequent observation in Addison's disease even after the restoration of electrolyte and water balance by appropriate therapy (2). Excessive treatment of patients suffering from Addison's disease with desoxycorticosterone acetate produces salt and water retention and later may lead to hypertension and cardiac failure (3, 4, 5).

The evidence that some product of the adrenal cortex plays a part in the mechanism of hypertensive vascular disease is less clear cut. Goldblatt (6) found that the development or maintenance of elevated blood pressure levels in dogs from constriction of both renal arteries appeared to depend upon the presence of adrenal cortical tissue. His findings have been substantiated by several investigators (7, 8, 9). Rogoff et al (10), on the other hand, reported the persistence of hypertension in a few totally adrenalectomized dogs without supportive treatment, and concluded that the adrenal gland does not play a significant role in experimental renal hypertension. Clinically, hypertension is usually present in Cushing's syndrome (11), now thought to be caused by adrenal cortical hyperplasia or tumor (hyperadrenocorticism) (12). Reduction in blood pressure levels of some patients with hypertension has been reported following rigid restriction of dietary sodium (13, 14, 15). Since the blood volume of hypertensive

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patients is relatively normal (16), this effect may be related to the observation that sodium chloride potentiates the pressor activity of desoxycorticosterone acetate (17, 18), and possibly of some naturally occurring cortical product as well

It was shown by Loeb and his associates (1) that adrenalectomized dogs excreted excessive amounts of sodium and chloride and they suggested that the so-called crisis in Addison's disease might result from a similar loss of these electrolytes Harrop and his co-workers (19) first observed that patients with adrenal insufficiency actually develop a reaction simulating a crisis of Addison's disease following the withdrawal of salt from the diet They proposed the use of a salt-free diet as a diagnostic test for Addison's disease Perera and Blood (20), using a similar test, found that patients with hypertensive vascular disease reacted to sodium restriction in the opposite fashion, salt and water were eliminated much less rapidly by such patients than by normal individuals In their experiment, twelve patients with hypertension showed a mean loss of body weight of only 0.4 percent, in contrast to a mean loss of 1.6 percent in twelve normotensive subjects following 24 hours of sodium withdrawal This difference was consistent, regardless of variations in environmental temperature and physical activity Significant changes in blood pressure levels or hematocrit were not observed after 24 hours of sodium restriction The investigators concluded that a disturbance in salt and water metabolism exists in hypertensive vascular disease as judged by the abnormal response to the abrupt and rigid restriction of sodium in the diet

The discovery of this apparently abnormal metabolic characteristic of hypertensive individuals immediately raises an important question: is the inability to lose weight promptly following sodium withdrawal a result, or a precursor of the hypertension? It would be of great interest if such a metabolic factor could be found which preceded the development of certain types of hypertension It was decided to subject a considerable number of healthy young adults to acute sodium withdrawal in order to ascertain the normal range of weight loss and to search for individuals with normal blood pressures whose response to sodium withdrawal was identical with that reported as characteristic of hypertensive subjects This experiment was conducted as part of

an extensive study of normal subjects in which the significance of certain hereditary, physiological and psychological factors as precursors of hypertension is to be evaluated

*Method* Observations were made on 83 male medical students in good health, who submitted to the dietary regimen while ambulatory and attending their regular courses. 64 of the 66 men in the first year class of the medical school participated in the experiment as part of their laboratory work in Physiology. In addition, 17 men in the fourth year class and 2 men in the third year class volunteered to take part in the study, bringing the total number up to 83 individuals. Women were not included in this preliminary study because of the phasic variation in body weight which occurs in relation to the menstrual cycle.

The general plan of the experiment was similar to that of Perera and Blood, the subjects were given a standard diet containing 1 gram of sodium chloride for three consecutive days to which 12 grams of salt were added on the first two days. On the third day, the additional sodium chloride was withdrawn, so that for 24 hours the subjects received a diet which was nearly sodium free. Body weight was determined each morning. No attempt was made to observe daily urinary output, hematocrit values or blood pressure levels, since consistent changes in these factors had not been previously reported, and to obtain valid data concerning them under the conditions of the experiment would have added enormously to its complexity.

Quarters were obtained where our staff had complete charge of preparing and serving the food. No other meals were served, thus eliminating the possibility of confusion between different diets simultaneously prepared. The standard diet as calculated contained 2900 calories, consisting of protein 86 grams, carbohydrate 394 grams, fat 109 grams, and approximately 1 gram of sodium chloride (Table 1). On the first two days six grams of sodium chloride were supplied in a shaker to each subject, to be completely used each day. In addition, he received 6 grams of sodium chloride in enteric coated tablets, two grams after each meal. On the third day the standard diet was served without additional sodium chloride. Thus the subjects received a normal or slightly high sodium diet for two days and an extremely low sodium diet on the third day, while caloric intake remained



constant The fluid intake of the first day was noted and kept approximately the same for each individual on the three days of the study, but no attempt was made to impose a standard 24 hour fluid intake on the group as a whole

The experiment was carried on for five weeks with 19 to 22 students undergoing the regimen each week <sup>2</sup> The students were weighed without clothes, after voiding and before breakfast Independent weigh-

TABLE I  
*Standard Low Sodium Diet*

BREAKFAST		LUNCH		DINNER		EVENING NOURISHMENT	
	Gms		Gms		Gms		Gms
Orange juice	120	Ground round	90	Pineapple	120	Jam sand-	
Puffed wheat	15	steak	150	juice		wich	
Egg (1)	50	Baked potato		Lean beef	100	Bread*	40
(soft or hard		Salad	75	steak		Butter*	5
boiled)		Tomato	20	Boiled new	100	Grape jam	25
Toast*	60	Lettuce	60	potatoes		Banana	150
Butter*	15	Bread*	20	Fresh peas	100		
Grape jam	25	Butter*		Celery hearts	10		
Cream		Canned		Radishes	10		
Heavy cream	30	peaches	150	Carrot strips	20		
Whole milk	90	Peaches	50	Bread*	60		
Coffee	120	Juice	180	Butter*	20		
Sugar	15	Coca Cola		Fresh straw-	100		
				berries			
				Sugar	10		
				Lemonade			
				Sugar	15		
				Lemon juice	15		

\* = salt free

ings of each student were made by two assistants, whose readings checked each other within 2 oz, with a mean deviation of 0.7 oz or 0.02 kg The average of the two readings was taken as the daily weight In addition to the weights, the record sheets included notations concerning fluid intake, number and time of bowel movements,

<sup>2</sup> In addition, a preliminary trial was carried out on Subjects 1 and 2, on the Metabolism Ward of the Johns Hopkins Hospital, through the kindness of Dr John Eager Howard and Miss A Elizabeth Crozier, Dietician

exact food consumption,<sup>3</sup> any slight symptom or deviation from health, and amount of smoking

An analysis of the sodium content of the standard diet was made each week on the day of sodium withdrawal<sup>4</sup> One-half of the total daily diet (as fed the subject) was carefully dried and ground to fine powder An aliquot was accurately weighed and ashed in a muffle furnace at 500° C in a platinum dish, then dissolved in distilled water to a volume of 100 ml, and analyzed in a flame photometer The sodium content per 24 hour total diet ranged from 16.7 to 25.1 meq, with a mean value of 20.2 meq The actual amount of sodium in the diet each week is shown in Table 2, expressed both in milliequivalents of sodium and as grams of sodium chloride

### RESULTS

A total of 107 tests were carried out on 83 subjects Fifty-nine students underwent the regimen once, while twenty-four subjects submitted to duplicate tests The data have been tabulated in Table 2 Eighty-one subjects lost weight in 103 tests following 24 hours of sodium restriction Subject 44 gained a very small amount in both of two tests, while Subject 7 lost a small amount on one test and gained a trifle on the other The range of weight change from Day 3 to Day 4 (the period of sodium restriction) was from -1.4 kg to +0.1 kg Since body weight ranged from 110.6 to 51.1 kg, it was considered desirable to calculate the weight change in terms of percentage loss of total body weight The range of percentage weight change was from -2.16 to +13% (all subjects)

64 of 66 men in the first year class participated in the experiment They may be considered as a satisfactory unselected sample of normal male medical students, ranging in age from 19 to 31 with a mean age of 24.2 years The curve of distribution (Figure 1) of the percentage weight change has been based upon the first trials of these 64 subjects

<sup>3</sup> If the subject failed to eat a portion of the diet (such as radish, tomato or evening nourishment) on the first day, the same item was omitted on the subsequent days

<sup>4</sup> The analyses were carried out with the assistance of Mr Harry Eisenberg, Chemical Division of the Department of Medicine

TABLE 2

EXPERI- MENTAL PERIOD	SODIUM CONTENT OF STANDARD DIET FOR 24 HOURS		SUBJECT NUMBER <sup>1</sup>	AGE	BODY WEIGHT IN KILOGRAMS					WEIGHT CHANGE FOLLOWING SODIUM WITHDRAWAL DAY 3-DAY 4	
	Na mEq	NaCl Gms			Day 1	Day 2	Day 3	Day 4 <sup>2</sup>	Day 5 <sup>3</sup>	Kg	%
I March 23-27	24 1	1 4	1 A	20	68 5	69 0	69 2	68 1		-1 1	-1 59
			2 A	22	56 6	56 7	56 7	56 1		-0 6	-1 06

mean = 62 55 62 85 62 95 62 10

II April 13-17	19 3	1 1	3 A	26	57 04	57 07	56 84	56 36	56 53	-0 48	-0 84
			4	28	72 74	72 60	72 09	71 30	71 74	-0 79	-1 10
			5 A	26	67 86	67 92	67 75	66 96	67 81	-0 79	-1 17
			6	20	68 32	67 55	68 03	67 15	68 04	-0 88	-1 29
			7 A <sup>4</sup>	22	68 83	67 75	67 61	67 69	67 47	+0 09	+0 13
			8 A <sup>4</sup>	22	60 33	60 64	60 58	60 27	59 79	-0 31	-0 51
			1 B	20	67 36	66 99	67 10	66 14	67 24	-0 96	-1 43
			9	24	59 76	59 45	59 82	58 80	59 25	-1 02	-1 71
			10	31	69 34	69 68	69 39	68 43	68 01	-0 96	-1 38
			11	31	65 77	65 77	66 05	65 46	65 66	-0 59	-0 89
			12 A	21	56 93	56 93	56 84	56 02	56 30	-0 82	-1 44
			13 A	22	73 14	73 59	73 70	73 42	73 46	-0 28	-0 38
			14	22	79 95	79 44	79 41	78 22	78 83	-1 19	-1 50
			15	26	71 78	71 89	71 66	70 61	71 30	-1 05	-1 47
			16	27	59 53	59 79	60 07	59 22	60 33	-0 85	-1 42
			17	21	72 97	72 71	72 91	71 69	72 83	-1 22	-1 67
			18	29	61 74	61 80	61 80	60 69	60 02	-1 11	-1 80
			19	23	59 47	59 47	59 50	58 71	59 56	-0 79	-1 33
			20 A	21	76 20	76 74	76 63	76 21	76 48	-0 42	-0 55
			21 A	30	65 60	65 09	64 64	63 76	64 07	-0 88	-1 36
			22 A	23	73 53	74 90	73 99	73 28	73 29	-0 71	-0 96
			23	21	74 84	75 10	74 90	74 05	74 80	-0 85	-1 14

mean = 67 41 67 40 67 33 66 57 66 95

III April 20-24	17 0	1 0	24 A	22	68 38	68 15	68 26	67 78	67 81	-0 48	-0 70
			25 A	24	67 02	67 13	67 13	66 56	66 91	-0 57	-0 85
			26 A	21	85 10	84 42	84 76	83 51	84 71	-1 25	-1 47
			27	21	72 69	71 84	71 53	70 68	71 50	-0 85	-1 19
			28 A	21	71 21	70 62	70 88	69 77	70 08	-1 11	-1 57
			29 A	22	66 05	66 11	65 54	65 26	65 19	-0 28	-0 43
			30 A	26	91 45	90 23	89 75	89 41	89 81	-0 34	-0 38

<sup>1</sup> A and B indicate two trials carried out on the same subject<sup>2</sup> At the end of twenty-four hours on low sodium diet<sup>3</sup> After 24 hours of diet and salt as desired<sup>4</sup> Upper classman, not included in distribution curve

TABLE 2—Continued

EXPERIMENTAL PERIOD	SODIUM CONTENT OF STANDARD DIET FOR 24 HOURS		SUBJECT NUMBER <sup>1</sup>	AGE	BODY WEIGHT IN KILOGRAMS					WEIGHT CHANGE FOLLOWING SODIUM WITHDRAWAL DAY 3-DAY 4	
	Na mEq	NaCl Gms			Day 1	Day 2	Day 3	Day 4	Day 5	Kg	%
III April 20-24 <i>Cont</i>			31 A	23	60 27	60 44	60 16	60 05	60 16	-0 11	-0 18
			32	25	76 09	75 81	75 41	74 79	75 69	-0 62	-0 82
			7 B <sup>1</sup>	22	69 00	68 49	67 53	67 25	67 02	-0 28	-0 41
			33	21	69 40	69 77	69 80	68 95		-0 85	-1 22
			34	23	80 85	80 43	80 17	79 35	80 40	-0 82	-1 02
			35	22	62 59	62 48	62 54	61 55	62 54	-0 99	-1 58
			36	22	74 78	74 61	74 95	74 16	74 39	-0 79	-1 05
			37	20	59 25	59 45	59 56	58 60	58 57	-0 96	-1 61
			38 A	19	53 64	54 09	53 83	53 55	54 09	-0 28	-0 52
			39	21	85 39	85 19	85 61	84 42	84 99	-1 19	-1 39
			40	23	64 52	64 78	64 78	64 33	64 52	-0 45	-0 69
			41 <sup>1</sup>	22	58 00	58 06	58 26	57 95	58 68	-0 31	-0 53
			42	23	72 85	72 26	72 54	71 32	72 23	-1 22	-1 68
			43 A <sup>1</sup>	23	77 16	76 82	76 78	76 20		-0 58	-0 76

mean = 70 75 70 53 70 47 69 78 69 96

IV April 27-30	16 7	1 0	44 A	21	109 46	108 18	107 64	107 73		+0 09	+0 08
			45	21	62 88	63 19	63 05	62 31		-0 74	-1 17
			46	25	59 87	60 04	59 95	59 50		-0 45	-0 75
			47	26	58 94	59 25	59 65	59 08		-0 57	-0 96
			48	22	69 45	69 65	70 02	68 97		-1 05	-1 50
			49	20	70 08	70 59	70 42	69 20		-1 22	-1 73
			50	24	50 66	50 92	51 09	50 13		-0 96	-1 88
			51	29	77 14	77 20	77 37	76 63		-0 74	-0 96
			52 A	29	92 79	93 13	93 07	92 93		-0 14	-0 15
			53	29	74 42	75 21	75 41	74 36		-1 05	-1 39
			54	25	62 74	63 16	63 05	62 03		-1 02	-1 62
			55	29	64 71	64 21	64 30	63 39		-0 91	-1 42
			56	29	105 60	105 23	104 81	103 87		-0 94	-0 90
			57	27	81 96	82 10	81 59	80 77		-0 82	-1 01
			58 A	21	68 26	68 12	67 58	67 13		-0 45	-0 67
			59	27	84 34	84 45	84 14	83 18		-0 96	-1 14
			60	30	80 23	80 09	79 92	79 07		-0 85	-1 06
			61	28	65 74	66 36	65 77	64 78		-0 99	-1 51
			62	30	71 52	71 55	71 52	70 64		-0 88	-1 23
			63	28	61 17	61 17	61 57	60 72		-0 85	-1 38
			2 B	22	54 48	55 76	55 14	53 95		-1 19	-2 16

mean = 72 69 72 84 72 72 71 92

TABLE 2—Concluded

EXPERI- MENTAL PERIOD	SODIUM CONTENT OF STANDARD DIET FOR 24 HOURS		SUBJECT NUMBER <sup>1</sup>	AGE	BODY WEIGHT IN KILOGRAMS					WEIGHT CHANGE FOLLOWING SODIUM WITHDRAWAL DAY 3-DAY 4				
	Na mEq	NaCl Gms			Day 1	Day 2	Day 3	Day 4 <sup>2</sup>	Day 5 <sup>3</sup>	Kg	%			
V May 4-7	19 1	1 1	64	20	68 49	68 38	68 61	67 70		-0 91	-1 33			
			24 B	22	67 53	67 53	68 12	67 75		-0 37	-0 54			
			25 B	24	67 73	68 15	67 81	66 85		-0 96	-1 42			
			65 <sup>4</sup>	22	67 30	67 53	67 98	66 90		-1 08	-1 59			
			29 B	22	66 39	65 77	65 18	65 07		-0 11	-1 17			
			58 B	21	67 27	67 47	67 50	66 59		-0 91	-1 35			
			38 B	19	53 66	54 43	54 57	54 17		-0 40	-0 73			
			28 B	21	71 16	70 45	70 19	69 62		-0 57	-0 81			
			66 <sup>4</sup>	22	71 32	71 55	71 07	70 22		-0 85	-1 20			
			3 B	26	56 02	56 36	56 73	55 96		-0 77	-1 36			
			5 B	26	67 73	67 90	67 59	67 02		-0 57	-0 84			
			8 B <sup>4</sup>	22	60 33	61 24	60 33	60 22		-0 11	-0 18			
			67 A	27	75 01	75 75	75 86	74 44		-1 42	-1 87			
			12 B	21	56 18	56 35	56 44	55 76		-0 68	-1 20			
			13 B	22	73 33	73 42	73 31	72 63		-0 68	-0 93			
			31 B	23	61 38	60 87	60 81	59 76		-1 05	-1 73			
			30 B	26	90 49	90 72	90 98	90 24		-0 74	-0 81			
			20 B	21	75 75	76 23	76 37	76 06		-0 31	-0 41			
			68 <sup>4</sup>	23	67 27	67 47	67 61	66 67		-0 94	-1 39			
			43 B <sup>4</sup>	23	76 99	76 71	76 71	75 75		-0 96	-1 25			
			52 B	29	93 61	93 58	92 90	92 36		-0 54	-0 58			
			69 <sup>4</sup>	24	82 16	82 19	81 82	81 08		-0 74	-0 90			
mean = 69 87 70 00 69 93 69 22														
VI May 11-14	25 1	1 5	26 B	21	86 35	85 22	85 22	84 43		-0 79	-0 93			
			22 B	23	74 11	74 73	74 79	73 74		-0 85	-1 14			
			70	26	59 42	59 99	59 88	59 11		-0 77	-1 29			
			71	20	74 50	74 44	73 99	73 82		-0 17	-0 23			
			67 B	27	74 84	75 32	75 46	74 44		-1 02	-1 35			
			44 B	21	113 39	111 29	110 64	110 73		+0 09	+0 08			
			72 <sup>4</sup>	23	74 22	74 73	74 16	73 59		-0 57	-0 77			
			73	24	72 52	73 17	72 91	72 12		-0 79	-1 08			
			74 <sup>4</sup>	22	87 77	87 09	86 92	86 41		-0 51	-0 59			
			75 <sup>4</sup>	23	72 77	72 86	72 77	72 00		-0 77	-1 06			
			76 <sup>4</sup>	20	67 27	68 04	68 38	68 04		-0 34	-0 50			
			77 <sup>4</sup>	26	59 59	59 36	59 64	59 10		-0 54	-0 91			
			78 <sup>4</sup>	22	71 58	71 89	71 72	71 21		-0 51	-0 71			
			79 <sup>4</sup>	24	72 40	72 88	72 91	71 86		-1 05	-1 44			
			80 <sup>4</sup>	21	78 07	78 58	78 30	77 93		-0 37	-0 47			
			81 <sup>4</sup>	24	78 07	78 10	78 07	77 53		-0 54	-0 69			
			21 B	30	64 52	64 26	64 49	64 35		-0 14	-0 22			
			82 <sup>4</sup>	23	72 46	72 86	73 40	72 92		-0 48	-0 65			
			83 <sup>4</sup>	23	76 03	76 03	75 46	74 78		-0 68	-0 90			
			mean = 75 26 75 31 75 22 74 64											

only<sup>5</sup> This curve shows that 46 of the 64 subjects lost between 0.90 and 1.89 percent of body weight, with the peak of greatest frequency at 1.4 percent. The distribution curve is skewed in the direction of lesser amounts of weight loss, so that while only one individual lost 1.9 percent or more of body weight, 17 subjects, or 26.6 percent, showed a weight change of less than -0.90 percent, ranging from -0.89 to +0.08 percent. Since none of Perera and Blood's 12 normotensive subjects lost less than 0.84 percent, and since the range of weight change of their series of 12 hypertensive patients was from -0.88 to +0.28 percent, it appeared important to consider further

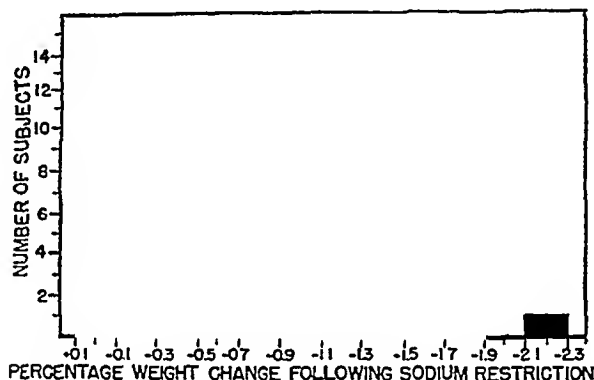


FIG 1 Distribution curve showing the percentage change in body weight following a 24 hour period of sodium restriction occurring among 64 unselected male students on their first tests

this significant group of our subjects whose weight response was similar to that of their hypertensive series (20)

It was evident that these 17 subjects might have lost less weight than the majority of their fellows following sodium withdrawal for one or more of the following reasons

1 The conditions of the experiment were not sufficiently rigid to prevent considerable physiological variability in response

<sup>5</sup> The data of the 19 upper classmen, important in certain regards, is not included in the distribution curve. They entered the experiment for a number of special reasons and so cannot be regarded as part of a "random sample", some had a family history of hypertension, high normal blood pressure or a positive cold test, while others partook because of their interest or accessibility

2 The subjects had in some measure failed to comply with the conditions of the experiment and had therefore not been exposed to the full effect of sodium withdrawal

3 The subjects actually were less physiologically responsive to sodium withdrawal than the larger portion of the group examined

The test was, therefore, repeated in 24 subjects, including 15 "small losers" who lost less than 0.9 percent body weight following sodium restriction on the first trial and nine "large losers" who lost 0.9 percent or more

The weight loss following the first and second trials of sodium withdrawal is recorded in Table 3, together with the percentage difference of each of the two tests from the mean. In 19 out of 24 individuals, or 79 percent, the difference between the mean and the actual tests was less than 0.3 percent of body weight. Thirteen of the fifteen "small losers" showed this high degree of consistency upon duplicate tests, and nine fell within the limits of the "small loser" group on both of the tests (Subjects 44, 20, 24, 38, 29, 8, 52, 30 and 7). Out of the entire 24, nine subjects fell on one test in the "small loser" and in the other test in the "large loser" group. The consistency of the test was good in four of the nine, suggesting that their responses were actually "borderline" (Subjects 43, 3, 13 and 25) while in five individuals (Subjects 58, 31, 28, 2 and 21) the degree of inconsistency suggested that some additional factors rendered the tests unsatisfactory. The possible nature of these factors will be considered presently.

In contrast to the nearly uniform weight loss following salt restriction, the daily weights fluctuated in *both* directions and to a lesser extent during the first 48 hours on the standard diet plus sodium chloride (Table 2). This is reflected in the mean values for the daily weights for each of the experimental periods. These daily mean values are plotted in Figure 2, which clearly illustrates the consistent trend toward decreased weight following sodium restriction. That the smaller daily variations in weight during the period of adaptation to the standard diet were not merely haphazard, but were related to the physiological makeup of the individual is suggested by the high degree of consistency of many of the duplicate tests (Figure 3). Such consistency was evident throughout the four day experimental period in Subjects 26, 67, 12, 43, 13, 44 and 20, while in Subjects 22, 28, 5, 1, 29

and 8, the correspondence of weights on three out of the four days was excellent. In still another group (Subjects 58, 7 and 38) the duplicate weight curves show considerable similarity. In only a few instances (Subjects 2, 21 and 30) were the two curves highly dissimilar. Since all but one (Subject 12) of the duplicate tests were carried out on subjects whose first test was unusual in some regard,<sup>6</sup> the most inconsistent results might be expected to appear among this group of

TABLE 3

*Percentage Weight Loss of Twenty-Four Subjects in Duplicate Salt Withdrawal Tests*

"SMALL LOSERS" IN FIRST TEST					"LARGE LOSERS" IN FIRST TEST				
Subject No	% Weight Loss		Mean % Wt. Loss 2 Tests	% Difference each Test from Mean	Subject No	% Weight Loss		Mean % Wt. Loss 2 Tests	% Difference each Test from Mean
	1st Test	2nd Test				1st Test	2nd Test		
44	+0 08	+0 08	-0 08	0 00	1	-1 59	-1 43	-1 51	0 08
20	-0 55	-0 41	-0 48	0 07	22	-0 96	-1 14	-1 05	0 09
24	-0 70	-0 54	-0 62	0 08	12	-1 44	-1 20	-1 32	0 12
38	-0 52	-0 73	-0 625	0 105	5	-1 17	-0 84	-1 005	0 165
29	-0 43	-0 17	-0 30	0 13	67	-1 87	-1 35	-1 61	0 26
8	-0 51	-0 18	-0 345	0 165	26	-1 47	-0 93	-1 20	0 27
30	-0 38	-0 81	-0 595	0 215	28	-1 57	-0 81	-1 19	0 38
52	-0 15	-0 58	-0 365	0 215	2	-1 06	-2 16	-1 61	0 55
43	-0 76	-1 25	-1 005	0 245	21	-1 36	-0 22	-0 79	0 57
3	-0 84	-1 36	-1 10	0 26					
7	+0 13	-0 41	-0 14	0 27					
13	-0 38	-0 93	-0 655	0 275					
25	-0 85	-1 42	-1 135	0 285					
58	-0 67	-1 35	-1 01	0 34					
31	-0 18	-1 73	0 955	0 775					

subjects. The fact that so many of these curves were repeatable attests to the general cooperation of the subjects and the validity of the method.

In experimental periods II and III (Table 2 and Figure 2) the subjects were allowed to return to their usual uncontrolled diets for 24 hours after the period of salt withdrawal and were then weighed under

<sup>6</sup> Subjects 1 and 2 weighed partially clad in first tests on Metabolism Ward. Subjects 5 and 22 irregular curves. Subject 21 continuous weight loss throughout first experimental period. Subjects 67, 26, and 28 unusually large weight loss on sodium withdrawal. Remaining 15 subjects "small losers".



standard conditions. The 37 first year students thus observed showed a mean weight gain of 60% of the weight lost while sodium was restricted at the end of 24 hours of unrestricted salt intake.

It is beyond the scope of this experiment to investigate in detail the various factors which might contribute to the daily fluctuations in weight while the subjects were on a standard diet, but brief statements may be made about a few of them. There is little evidence that changes in caloric intake played a significant role. All subjects found the diet satisfactory and quantitative differences from their customary

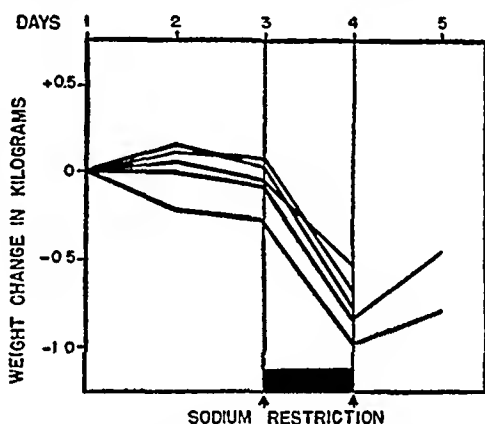


FIG 2 THE EFFECT OF SODIUM RESTRICTION UPON BODY WEIGHT IN 105 TESTS

Each line represents the mean weight of all the subjects taking part in one experimental period. (See Table 2 for detailed data. Period I, a preliminary study on 2 subjects only, is not included.)

food intake probably were seldom great. Consistent weight change in one direction or the other throughout the preliminary 48 hours was not conspicuous in most subjects. The heaviest subject in the study, however, was an outstanding exception in this regard.

Subject 44, who is 6'1" tall, weighed 121.6 Kg 6 months before taking part in the experiment. On a 1700 calorie reducing diet without salt restriction his weight fell to 105.2 Kg at an average rate of 0.22 Kg lost per day. During the period of reduction he was weighed frequently, the most rapid weight loss was at a rate of 0.48 Kg per day for one 7 day period. Strict dieting was abandoned 11 weeks before the experiment began and weight had increased to 109.5 Kg. Between Test A and B it increased still further to 113.4 Kg. In the first 48 hours of

the experiment, he lost weight at a rate of 0.95 Kg and 1.4 Kg per day during tests A and B respectively, a much more rapid rate than when he was on the reducing diet. Salt intake seemed to him a little higher than normal during these two days. On the same caloric intake, weight loss ceased when salt was withdrawn (Figure 3). This response remains to be explained. It suggests that the usual type of salt and water control was modified in this obese subject.

Spontaneous comments were recorded on the quantity of salt ingested with the standard diet on Days 1 and 2 in comparison with the subjects' usual salt intake. The mean daily weights of the subjects who believed that salt intake was unusually *high* were compared with those who considered salt intake unusually *low* during that period. No significant change in weight at the end of 48 hours was present in either group.

The only subject for whom an exception was made in regard to salt intake was as follows:

Subject 61 stated that he was in the habit of eating large quantities of salt, up to 40 grams daily. Examination revealed normal blood pressure and no clinical evidence of Addison's disease. During Days 1 and 2 he was allowed to use as much salt as he pleased from the shaker, and actually consumed 29 grams of shaker salt plus 6 grams of sodium chloride in tablet form daily. He weighed 65.74 Kg on Day 1, and 65.77 Kg after 48 hours on this unusually high salt intake. On Day 3 he ate the same sodium-restricted diet as the others. Following sodium withdrawal, he lost 1.51 per cent of body weight, thus conforming closely to the mode of 1.4 per cent for the unselected group.

Body weight was not consistently affected by variations in bowel activity except when stools were totally absent for 24 hours (Figure 3). When a subject had *no* bowel movement on a certain day in one test and *one* or *more* movements on the corresponding day in the duplicate test, the body weight was always higher, as might be anticipated, when no bowel movement had occurred (Subjects 22, 28, 2, 21 and 3). Some of the more obvious discrepancies in duplicate tests were unrelated to the number of stools per day (Subjects 5 and 8), and conversely, rather different numbers of bowel movements in 24 hours did not necessarily interfere with the similarity of the duplicate curves (Subject 20).

Smoking was not restricted during the experiment. Thirty-five subjects, however, did not smoke at all during the tests. Fourteen

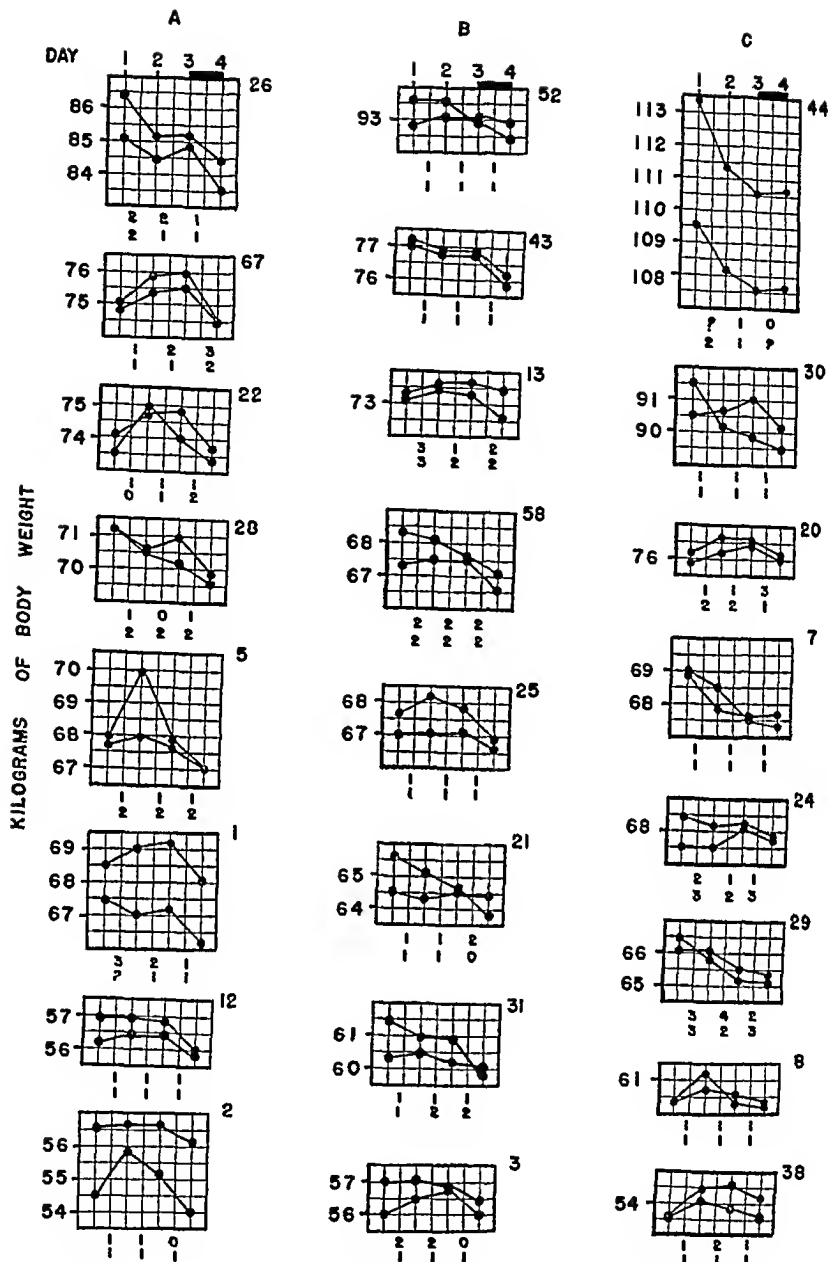


FIG 3 THE EFFECT OF SODIUM RESTRICTION UPON BODY WEIGHT  
IN TWENTY-FOUR DUPLICATE TESTS

indicates 24 hour period of sodium withdrawal from Day 3 to Day 4

The subject numbers are given to the right of each pair of curves, for further details, see Table 2. Note that the upper curve of each pair represents *heavier weight* and is not necessarily the first test. The number of bowel movements for each 24 hour period are charted below each pair of curves.

Column A subjects who were "large losers" on both tests

Column B subjects who were "large losers" on one test, and "small losers" on the other

Column C subjects who were "small losers" on both tests

subjects smoked a little, 15 smoked from 10 to 19 cigarettes a day, and 19 smoked 20 cigarettes a day or more. "Large Losers" and "small losers" appear in all the groups, and there is no indication that smoking affected the weight loss following sodium withdrawal.

Despite efforts to exclude subjects from participating when any ailment was present, six (Subjects 26, 7, 31, 46, 73 and 74) developed fresh upper respiratory infections during the test period. Three of

TABLE 4  
*Response to Sodium Withdrawal during Upper Respiratory Infection*

SUBJECT NO	TEST NO	% WEIGHT LOSS AFTER SODIUM WITHDRAWAL	
		Infection Present	Infection Absent
7	A	+0 13	
	B		-0 41
26	A		-1 47
	B	-0 93	
31	A	-0 18	
	B		-1 73
Mean		-0 33	-1 20
46		-0 75	
73		-1 08	
74		-0 59	
Mean of six tests, "Infection Present"		-0 57	Range, -1 08 to +0 13

these had duplicate tests when they were free from infection (Table 4).

It will be noted that in all three subjects who had duplicate tests weight loss was less during the test in which infection was present. While the instances of upper respiratory infection were so few that no conclusions can be drawn, the data raise the possibility that sodium withdrawal during this type of infection results in a subnormal loss of body weight.

*Discussion and Correlations* Of 64 unselected students, 47 lost 0.9 percent or more of body weight following sodium restriction. Seventeen, however, lost less than this amount, and in this respect

resembled the hypertensive patients studied by Perera and Blood (20) Where duplicate tests were done, they were consistent in 79 percent of cases The data show that a considerable porportion of normal subjects had the so-called hypertensive pattern of response to sodium restriction

May this pattern of response, when found in a normal individual, indicate a "prehypertensive state"? This can only be directly determined by observing such subjects over a period of years Indirect evidence, however, may be sought through evaluating a number of factors which have been found by other investigators to show a positive correlation with the subsequent development of hypertension These include familial hypertension, overnutrition, high normal blood pressure levels and vasomotor hyperreactivity to the cold pressor test Did those of our normal subjects who presented one or more of these four traits show a different pattern of weight change following sodium withdrawal from the subjects who did not show any of these traits?

In order to determine whether any such correlation existed, the subjects were separated into groups according to the following categorical criteria

- 1 *Familial hypertension* history of definite hypertension in one or both parents (21)
- 2 *Overnutrition* 10 lbs or more above "ideal" body weight for the subject's height and age (22)
- 3 *High normal blood pressure levels* resting levels above 120 mm systolic and/or 80 mm diastolic pressure
- 4 *Vasomotor hyperreactivity to the cold pressor test*<sup>7</sup> a rise of 20 mm or more of systolic and/or diastolic pressure
- 5 *None of these Four Traits*

The authors are fully aware of the theoretical difficulties inherent in any such arbitrary division, these will be discussed in subsequent papers in which each one of these factors is analyzed in detail, together with the methods used It is obvious, for instance, that group 1 does not include all those with familial hypertension, while group 2 includes some who have no excess fat Nevertheless, by this means a practical sorting out of groups which present different characteristics is accomplished

<sup>7</sup> Method of Hines and Brown (23)

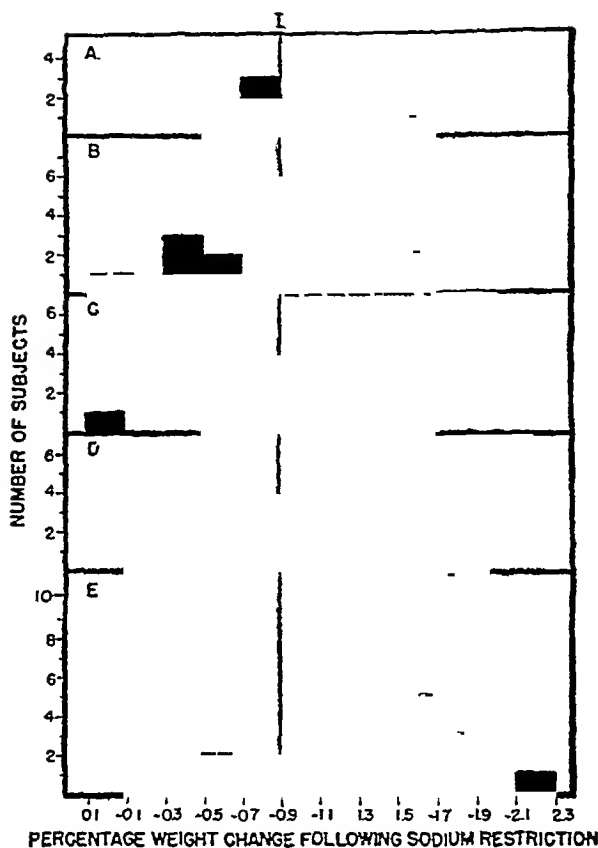


FIG 4 Distribution curves showing the percentage change in body weight following a 24 hour period of sodium restriction in

A 11 Subjects with Familial Hypertension

B 22 Subjects with Overnutrition

C 14 Subjects with High Resting Blood Pressure Levels

D 21 Subjects with Vasomotor Hyperreactivity

E 30 Subjects without these Four Traits (A, B, C and D)

I Indicates the borderline between the hypertensive and normal subjects of Perera and Blood

For this purpose, the 64 unselected subjects were divided into 30 who had none of the four traits and 34 who had one or more such traits, to the latter group were added the 12 upper classmen who were in-

cluded in the study because they showed one or more of these traits. There was some overlapping, while 27 subjects showed one trait only, 16 showed two and 3 had three of the traits defined above.

The distribution curves of the response of body weight to salt withdrawal of the subjects in each of these five groups is shown in Figure 4. The line of demarcation between the metabolic response of the hypertensive and the normal subjects of Perera and Blood has been used as a point of reference. It will be noted that a higher proportion of the subjects with familial hypertension, overnutrition, high normal blood pressure levels or vasomotor hyperreactivity show a metabolic response like that of hypertensive patients than occurs among subjects free from these four traits, although the numbers are not sufficiently large to make this difference statistically significant.

### *Summary and Conclusions*

1 The body weights of 83 male medical students were observed following twenty-four hours of rigid sodium restriction while on a standard diet.

2 The range of weight change following sodium restriction, expressed in percentage of total body weight, was from  $-2.16\%$  to  $+0.13\%$ .

3 In a group of 64 unselected students, a weight loss of  $1.4\%$  was the most frequent change observed.

4 The weight loss of 47, or  $73.4\%$  of these unselected subjects, fell within the range heretofore described as the "normal" response to sodium withdrawal.

5 The remaining 17, or  $26.6\%$  fell outside of that range, and coincided with the range previously considered characteristic of patients with hypertension.

6 Consistent results were obtained in 19 of 24 subjects who submitted to duplicate tests.

7 Our findings indicate that a considerable proportion of normal young men respond to sodium withdrawal in a manner similar to the response of hypertensive patients.

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# BRONCHOGRAPHY IN THE SEVERELY DISCHARGING LUNG

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Adequate bronchography in the presence of copious pulmonary discharge poses a perplexity of major import in the study of the bronchiectatic patient. Of the varied techniques designed to improve the efficiency of the bronchographic examination, all have demonstrated serious limitations in the type of case under discussion. This study concerns the occasional patient who expectorates in the neighborhood of 400 cc of secretion daily.

Following bronchoscopy, the sputum produced overnight in these cases prevents adequate opacification of even the major bronchi with contrast agent. If bronchoscopy immediately precedes the instillation, profound cough results in expectoration of the injected iodized oil. Anesthesia proves ineffectual, due to lack of contact of the local agent with the pus-lined bronchial walls. Additional instillations of anesthetic solutions also fail to overcome the irritation of bronchoscopic trauma. When aerosols of penicillin and/or streptomycin are employed, the quantity of discharge is materially lessened. Their use is contraindicated, however, in a purely diagnostic procedure, because of the likelihood of rendering the existing organisms drug-fast.

Although catheter suction of the tracheobronchial tree was found to be significantly less traumatic than bronchoscopy in our series, the results were inferior. Spasmodic closure of the glottis during coughing exhausts the air in the tree and the general negative pressure prevents point suction at the end of the catheter. This procedure cannot be employed in small children as sudden lowering of oxygen tension may quickly prove fatal.

Analysis of the results of the catheter method, nevertheless, indicated potentialities in this direction. To perfect this technique, increase in efficiency was required. Our problem resolved to maintenance of constant patency of the glottis—temporary exteriorization of the trachea, without resorting to the drastic procedure of tracheotomy.



FIG 1 Post operative pneumectomy for unilateral cystic disease. Conventional bronchographic study performed after bronchoscopy failed to provide explanation of persistent copious pulmonary discharge.



FIG 2 After aspiration of tracheobronchial tree by method described, true state of the long diseased left bronchial stump is demonstrated.

A new thin-walled, plastic endotracheal tube proved to be the solution to this enigma. It softened in warm water, on cooling it assumed a semi-rigid nature, elastic enough to prevent trauma. After anesthetizing the upper air passages, including the nares and nasopharynx, the tube was passed under mirror vision into the upper trachea, well above the carina. Both sides of the bronchial tree were aspirated by long catheter or small caliber plastic tubing through the lumen of the endotracheal tube. If the secretions were found to be tenacious, flushing with small quantities of saline solution was next performed. After removal of the pus, the lower tree was anesthetized by dilute pontocaine instillation. The agent was undoubtedly much more effective on the clean bronchial walls.

If the endotracheal tube were left in place, the oil catheter could be easily introduced through it into the tree. By not removing the tube until after the roentgenological examination was completed, coughing was reduced, and working in the darkened fluoroscopy room was facilitated. All of the cases studied were subjected to bronchoscopy twenty-four hours prior to bronchography for removal of collected discharge. Postural drainage was instituted after this procedure.

By this method, we have been able to demonstrate bronchiectasis in all five lobes and lingula at one bronchographic examination in a 64 year old female whose sputum was in excess of 800 cc per day. In another case of severe unilateral disease, producing nearly 1200 cc per day, we proved the contralateral lung to be essentially normal, and this cleared the patient for surgery. A case of cystic pulmonary disease with persistent post-operative discharge was shown to have a long diseased bronchial stump responsible for the continuance of symptoms (Fig 1 and 2). All other methods had failed to adequately demonstrate the bronchial tree in the cases cited above. We feel that this procedure is a valuable addition to the armamentarium of those attempting bronchography in the exceptional case of severe pulmonary discharge.

# PYRIDINE LIVER AND KIDNEY INJURY IN RATS, THE INFLUENCE OF DIET, WITH PARTICULAR ATTENTION TO METHIONINE, CYSTINE, AND CHOLINE<sup>1</sup>

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The development of necrosis of the liver, involving predominantly the central portions of the lobules, followed by cirrhosis, calcification, and large regenerative tumor-like nodules, in rats fed synthetic diets containing pyridine, has been described in previous reports (Baxter, '46, '48a). Obstruction to the flow of blood through the sinusoids accompanied, or possibly preceded and initiated, the development of necrosis, and death in some cases appeared to be the result of shock, due in part to trapping of blood in the liver (Baxter, '48b). Renal damage of severe degree frequently developed along with, or more likely following, the liver injury (Baxter, '48a, '49). Further investigations of the pathogenesis and relationship of the hepatic and renal lesions, with particular attention to the early changes, are now in progress.

It seemed in the beginning that the damage produced by pyridine might be due to a drainage of methyl groups from choline and methionine, which are important sources of labile methyl groups for biological methylations (du Vigneaud, '41), and it was observed that the injury was prevented to a considerable extent by supplements of methionine (Baxter, '47b). However, further studies made it appear very unlikely that pyridine injury was principally a result of methyl deficiency, or that the protection afforded by methionine was due to the lipotropic action which this amino acid has by virtue of its methyl groups (Baxter and Mason, '47). Actually, as will be discussed in more detail later, the morphological characteristics of the lesions at-

<sup>1</sup> The experimental work was done in part in the Department of Biochemistry, Cornell University Medical College, and in the Hospital of the Rockefeller Institute for Medical Research, New York City.

<sup>2</sup> Welch Fellow in Internal Medicine of the National Research Council.

tributable to pyridine were not, for the most part, suggestive of choline or labile methyl deficiency. The observations on the effects of pyridine itself, and of the dietary supplements when added to pyridine-containing diets, have recently been confirmed by Coulson and Brazda ('48).

Continued growth, during the time of development of liver and kidney damage, of the animals on the pyridine-containing diets with the higher protein levels, was considered evidence against the production of a deficiency or a "conditioned" deficiency of the sulfur-containing amino acids, of sufficient severity to account for the injury produced (Baxter, '47b). The role of substances containing sulfhydryl groups in protection against toxic substances which inactivate certain enzyme systems, and the possibility that the protective effect of methionine was through this mechanism, were considered (Baxter and Mason, '47).

During the course of the experiments utilizing pyridine-containing diets, it was observed that liver and kidney injury and animal mortality were much greater when pyridine was fed in the diet than when it was administered separately in daily or twice daily injections (Baxter, 48a). Death of some animals within 2 to 3 days after starting the pyridine-containing diets argued against the possibility that pyridine injury was due alone to the destruction of essential dietary factors.

With the object of learning more of the nature of the injury produced by pyridine, and of the relationship of methionine, cystine, and choline in protection against the injury, it was decided to make a detailed study of the effects on the injury produced, of adding supplements of these factors to pyridine-containing diets. Certain characteristics of the pyridine injury suggested that an appropriate situation for evaluating the possible modifying effects of various substances and procedures on the hepatic necrosis might be provided by the use of this agent, and it was hoped on the basis of the hypothesis that similar types of liver injury produced by a variety of means may result from disturbances of common labile mechanisms, that the information obtained might be applicable not only to pyridine injury, but also to comparable injury occurring under a wider range of circumstances.

The metabolic relationships of methionine, cystine, and choline are complicated and not completely understood. Methionine is an essen-

tial amino acid which ordinarily must be provided in the diet. Neither the methyl group nor the remaining part of the molecule can be synthesized in the body (du Vigneaud, '42). Cystine (Tarver and Schmidt, '39) and choline (du Vigneaud, Chandler, Cohn, and Brown, '40), on the other hand, may be formed in the body by the utilization of the sulfur and the methyl group of methionine, respectively, and additions of these substances may therefore spare dietary methionine under certain conditions.

In spite of the ability of the body to utilize the methyl groups of methionine in the synthesis of choline, a severe choline deficiency, as evidenced by the occurrence of liver and kidney lesions which are preventable by choline, may exist with fairly high levels of methionine in the diet, and raising the dietary methionine level under certain circumstances may actually exaggerate, rather than lessen, the degree of choline deficiency. This fact was illustrated by the effects on the incidence of the hemorrhagic-kidney syndrome of choline deficiency, of progressively increasing the casein (with its sulfur-containing amino acids predominantly in the form of methionine) level of the diet (Griffith, '41, Mulford and Griffith, '42). A number of observations have strongly suggested, as might be expected, that the amount of methionine available for choline synthesis in the body bears a reciprocal relationship to the amount of methionine utilized in tissue formation and growth, which in turn is influenced by the dietary content of other essential amino acids (Treadwell, Groothuis and Eckstein, '42, Beveridge, Lucas and O'Grady, '44, Treadwell, Tidwell and Gast, '44, Engel, '47). Some evidence from growth experiments suggests further that the methyl group is not available from that part of the dietary methionine which is converted to cystine and utilized in the body in that form (Mulford and Griffith, '42).

In addition to the relationships which depend on the ability of the body to utilize methionine in the formation of cystine and choline, it has frequently been observed that the choline requirement is, to a considerable extent, determined by the cystine content of the diet (Griffith and Wade, '40, Beeston and Channon, '36, Griffith, '40), or perhaps by the dietary cystine plus that portion of the dietary methionine which is converted to cystine. Mulford and Griffith ('42), however, have attributed this increase in choline requirement produced

by cystine to a general stimulation of metabolism by cystine, not proportional to the amount of cystine added, rather than to a direct antagonism between cystine and choline, and it is well established that the injury produced by high levels of cystine is not completely counteracted by choline (Earle and Victor, '42)

The minimum optimal dietary concentrations of methionine, cystine, and choline, even under normal conditions, can be determined only in a relative and restricted sense, and the theoretical difficulties of evaluating possible protective effects of the factors are apparent. If the diet contains sub-optimal amounts of one of the factors, then supplements of that factor might produce a protective effect due simply to a correction of the deficiency, whereas if excessive amounts are present from the beginning, a protective effect of the supplement might be masked by the amounts already present. It was in an effort to make apparent any such fallacies as these that four different basic diets were used in the present experiments. Furthermore, in order to rule out the possibility that effects of the supplements which were observed were due to modification by the supplements of the level of food and pyridine intake, it was necessary to measure accurately the food consumption of the animals of the various groups, and to control the food consumption in some of the experiments.

#### METHODS AND MATERIALS

##### *Animals*

Young male rats of various ages and strains were used in the experiments, which in most cases were continued for a month or longer. Further data concerning the animals used in each experiment are given in table 2. Prior to starting the experiments the animals were fed a stock diet<sup>3</sup>. Rats from common pools were distributed equally among the control and experimental groups. The animals of the various groups in each experiment were comparable in age, size, strain, and in previous treatment. The animals were not extensively studied in regard to intercurrent diseases. Diarrhea, vertigo, respiratory distress, and enlargement and darkening of the spleen, pulmonary suppuration, and small foci of necrosis in the liver were occasionally observed in-

<sup>3</sup> The rats employed in the experiments summarized in charts 1 and 2 were fed Rockland rat diet, while those of other experiments received Purina fox chow.



dividually or in combinations in control animals. Death or serious illness in control animals was rare but was occasionally noted. No extensive liver or kidney lesions, and no lesions having the character-

TABLE I  
*Composition of Experimental Diets*

	NO 1	NO 2	NO 3	NO 4
	%	%	%	%
Casein <sup>a</sup>	10	18	25	20
Lard	20	20	—	—
Hydrogenated vegetable oil	—	—	19 <sup>b</sup>	19 <sup>c</sup>
Corn oil <sup>d</sup>	—	—	1	1
Sucrose	30	22	50.8	55.5
Corn starch <sup>e</sup>	29	29	—	—
Salt mixture <sup>f</sup>	4	4	4	4
Yeast	5	5	—	—
Cod liver oil	2	2	—	—
L-cystine supplement	—	—	—	0.2
Choline chloride supplement	—	—	0.2	0.3
Vitamin supplements	—	—	+	+

Vitamin supplements per kilogram of Diets 3 and 4

Thiamine chloride	20 mg	Biotin	0.1 mg
Pyridoxine	20	Folic acid	0.2
Riboflavin	30	2-Methyl-1,4-naphtho-	
Ca pantothenate	50	quinone	1.5
Nicotinic acid	200	dl- $\alpha$ -Tocopherol	
Inositol	500	tate	15.0
Ascorbic acid	500	Vitamin A conc	60,000 U.S.P. units
Para amino benzoic acid	10.0	Vitamin D conc	6,000 U.S.P. units

<sup>a</sup> The casein used in diets 1 and 2 had not been extracted. Vitamin test casein (General Biochemicals, Inc.) was used in diets 3 and 4.

<sup>b</sup> "Covo"

<sup>c</sup> "Crisco"

<sup>d</sup> "Mazola"

<sup>e</sup> "Argo" corn starch

<sup>f</sup> Osborne and Mendel #2

istics of those attributed to pyridine, were ever observed in control animals. Results with different strains of rats were apparently quite uniform. The effects of pyridine in other animal species have not been investigated.

*Experimental Diets*

The composition of the four basic diets, some of which have been described previously (Baxter, '48a), are shown in table 1. Diets 1 and

2 contained only 250-300 mgm of choline chloride per kilogram of diet, and the casein levels were not sufficient to supply optimum amounts of the sulfur-containing amino acids. Diet 3 contained apparently optimum amounts of choline, and the casein level was sufficiently high that the gain in weight per gram of food on the diet was not increased by adding methionine. Diet 4 also contained at least optimum amounts of choline and the sulfur-containing amino acids, and was employed because of the objections to diet 3 which were previously discussed (Baxter, '47b).

The concentrations of pyridine citrate<sup>4</sup> and of the dietary supplements, which alone and in combinations were added to the basic diets, are indicated in table 2.

### *General plan of experiments*

In experiments employing each of the four basic diets individually, comparable groups of rats were transferred from the stock diet to each of the following regimens *ad libitum*: 1) basic diet alone, 2) basic diet plus methionine, 3) basic diet plus pyridine citrate, and 4) basic diet plus pyridine citrate plus the various dietary supplements, singly and in combinations.

The experimental diets prepared from each basic diet were fed simultaneously and under the same conditions, and the effects on health, growth, and survival of the animals were noted. The rats were housed in individual cages with large-mesh screen bottoms, and weighed at intervals of 1 to 3 days. The daily food consumption was followed in animals of many of the groups. Autopsies were performed on the animals which died, and sections of livers and kidneys were examined microscopically in a great many cases. The probability that some of the experiments were terminated too early to show the maximum difference in survival among the different groups, is evident from inspection of the charts. The experiments were terminated because of the interest in the morphological changes which occurred in the liver.

<sup>4</sup> Purchased from A. D. Mackay Co., New York City. The manufacturing chemist reported that this substance was prepared from equimolar quantities of pyridine and citric acid, which were allowed to react in warm alcoholic solution. The product was allowed to crystallize and was washed with absolute alcohol. A Kjeldahl determination done on the compound as it was used in the diets, without drying, showed 4.6% N, compared with a theoretical value of 5.17% N for the monopyridine salt.

and kidneys on the various modifications of the diets, observations on the frequency and degree of liver damage in the surviving animals, as well as the observations on the length and rate of survival in the various groups, were taken into consideration in formulating the conclusions

TABLE 2  
*Experimental Conditions Employed with Various Basic Diets*

	ANIMALS		CONCENTRATION OF PYRIDINE CITRATE (P)	CONCENTRATION OF SUPPLEMENTS			
	Initial weight	Strain		Methionine (M)	Cystine (Cy)	Choline (Ch)	Thiouracil <sup>e</sup> (Th)
	Gm		%	%	%	%	%
Diet 1 (Chart 1) <sup>a</sup>	Majority 50-80	Piebald animals of mixed strain	0.34	0.5	0.4	0.3-2.0	0.2
Diet 2 (Chart 2)	50-80	Sprague-Dawley	0.7	1.0	0.8-1.6	1.0	0.3
Diet 3 (Chart 3) <sup>b</sup>	Comparable groups 80-250	Sherman	1.0	1.0	0.8	1.0	0.2
Diet 4 (Chart 4) <sup>c</sup>	40-60	Sherman	1.0	1.0	—	—	—
Diet 4 (Chart 5)	40	Sherman (different source)	1.0 (Inj) <sup>d</sup>	1.0	—	—	—

<sup>a</sup> Growth curves of similar animals on diet 1, with and without pyridine, have been published previously (Baxter, '47a)

<sup>b</sup> Data on food consumption of control animals and animals given supplements of methionine are shown in table 4

<sup>c</sup> See table 3 for data on food consumption and growth of some of the animals of this experiment

<sup>d</sup> Pyridine by injection as described in text

<sup>e</sup> Results with thiouracil are included in the charts, see footnote 3 for discussion of preliminary results with substances other than methionine, cystine, and choline

The influence of methionine supplements on the injury produced by diets containing pyridine citrate was further studied in experiments utilizing paired-feeding techniques. In the first and second of these experiments, employing diets 3 and 4 respectively, animals of the groups with and without methionine supplements were paired, and the animals not receiving the added methionine were allowed to eat

*ad libitum* The twins receiving the methionine supplements were then offered equivalent amounts of food on the following day. Since some of the animals of the latter groups failed at times to eat all of the food offered, the pairing was not entirely satisfactory. In the third experiment, the food of all animals was arbitrarily limited to 32 grams, placed in the cages at the same time each day. A similar technique was successfully employed in previous experiments with pyridine-containing diets (Baxter, '48a).

The influence of different levels of consumption of pyridine-containing diets on survival of the animals was noted in the experiments with *ad libitum* feeding and was specifically studied in one experiment with controlled food intake.

Advantage was taken of the opportunity afforded by the paired-feeding experiments to compare again the toxicity of pyridine fed in the diet, with that of equivalent amounts administered by daily injection.

#### RESULTS WITH AD LIBITUM FEEDING

##### *Effects of basic diets alone*

Almost all of the animals receiving the basic diets alone, without added pyridine or dietary supplements, survived and remained well throughout the experimental periods. Some animals on diet 1, and less frequently on diet 2, developed fatty changes in the liver, but the changes were usually not extensive and usually did not result in fibrosis during the experimental periods. Effects of certain of the diets have been described in more detail elsewhere (Baxter, '47a, Baxter, '48a). Food intake, growth rate, and gain in weight per gram of food were considerably less on diet 1, and somewhat less on diet 2, than on diets 3 and 4.

##### *Effects of basic diets plus supplements of methionine*

Additions of supplements of DL-methionine (of the same sizes indicated in table 2) resulted in an increase in food consumption, growth rate, and gain in weight per gram of food with diet 1, and to a much lesser extent with diet 2. With diets 3 and 4, the average food consumption and growth rate remained the same as without the supplement, or increased slightly, while the gain in weight per gram of food remained

approximately constant (table 3) A few of the animals receiving methionine supplements, particularly with diet 4, developed diarrhea

TABLE 3

*Effects of Supplements of DL-Methionine and Pyridine Citrate on ad libitum Food Consumption and Growth on Diets 3 and 4*

	DIET 3	DIET 3 PLUS 1% METHIO- NINE	DIET 4 WITH OUT CYSTINE	DIET 4	DIET 4 PLUS 1% METHIO- NINE	DIET 4 PLUS 1% PYRI- DINE CITRATE	DIET 4 PLUS 1% PYRIDINE CITRATE PLUS 1% METHIO- NINE	DIET 4 PLUS 1% PYRI- DINE CITRATE	DIET 4 PLUS 1% PYRIDINE CITRATE PLUS 1% METHIO- NINE
						Series A	Series A	Series B <sup>a</sup>	Series B <sup>d</sup>
Initial average body weight, gms	43 3	44 5	43 7	44 9	45 0	45 7	45 1 (44 7)	50 0	53 0 (52 2)
Average gain in weight during first 2 weeks on diets, gms	43 2	49 3	38 0	43 3	40 6	35 8	33 7 (38 3)	28 5	22 1 (27 8)
Average food consumption during first 2 wks on diet, gms	82	93	81	88	84	81	81 (86)	69	62 (72)
Gain in weight per gram of food, gms	0 53	0 53	0 47	0 49	0 49	0 44	0 42 (0 45)	0 41	0 36 (0 39)
No of animals included in calculations	6	5	6	6	5 <sup>a</sup>	3	5 <sup>b</sup> (3) <sup>c</sup>	7	11 (7) <sup>c</sup>
No animals excluded because of death during experimental period	—	1	—	—	1	3	1	6	2

<sup>a</sup> One of these animals ate and grew poorly

<sup>b</sup> One of these animals developed diarrhea

<sup>c</sup> The figures enclosed in parentheses above were calculated on the basis of the number of animals indicated here, exhibiting the best growth

<sup>d</sup> This series of animals ate poorly at the beginning of the experiment

The data in this table were obtained in part from the experiment summarized in chart 4

or died Whether methionine was responsible to some extent for this morbidity and mortality, was not determined

*Effects of basic diets plus pyridine citrate*

The addition of pyridine citrate to the basic diets caused some decrease in food consumption and growth rate on all of the diets. This effect was so great with diet 1 that the animals often ceased to grow or even lost weight (Baxter, '47b), while with diets 3 and 4, average food intake, growth rate, and gain in weight per gram of food were much less substantially decreased, and sometimes they were almost as great as with the basic diets alone (i.e., the animals receiving 6.4 grams of pyridine-containing diet, chart 5). The effect of additions of pyridine on the efficiency of utilization of the food at any given level of food intake was not specifically studied, though on diets 3 and 4 where animals with and without pyridine sometimes consumed comparable amounts of food, it appeared that there was no great difference in rate of growth in the two groups prior to development of illness in the animals receiving pyridine.

After eating the diets containing pyridine citrate for several days or more, some of the animals began to drink larger amounts of water than normal and to excrete correspondingly large volumes of urine. A more ominous sign was a gain in weight out of proportion to the amount of food consumed, which was most readily detected in animals fed the same measured amount of food daily, and which was apparently due to the development of anuria or oliguria with abnormal retention of fluid. During this stage, the scanty urine often contained high concentrations of protein, in most cases neither red blood cells nor hemoglobin was observed in the urine, though pigment casts were occasionally seen in the renal tubules. At the same time, the animals often appeared pale, with little blood in the vessels of the ears, tail and skin, and sometimes felt quite cold to the touch. Blood was sometimes passed by rectum, perhaps as a result of portal congestion. Excessive tearing and passage of fluid from the nose not infrequently occurred, and the perineal region was often wet with urine. Such animals often died within 24 hours, but some of them in milder degrees of shock rallied and lived for longer periods. It appeared that renal injury of considerable degree usually occurred only following hepatic injury, and that it was caused in part by shock which often accompanied the hepatic injury (Baxter, '49). When hepatic injury was prevented, the renal injury did not occur. The renal injury differed from that oc-

curing in the hemorrhagic-kidney syndrome of choline deficiency (Baxter, '47a) It occurred in adult as well as young rats, and was not influenced by choline Lesions of the livers and kidneys similar to those already described (Baxter, '48a, '48b) were found in the animals which died, and in some of those which were sacrificed for autopsy (figures 1 and 2)

TABLE 4

*Effects of Adding Methionine to Diet No 3 Containing Pyridine, on Daily ad libitum Food Consumption During First 10 Days, and on Length of Survival of Animals*

DIET NO 3 PLUS	RAT NO	INITIAL WEIGHT OF ANIMALS	FOOD CONSUMED DURING SUCCESSIVE 24 HR PERIODS												PERIOD OF SURVIVAL OF ANIMALS
			1	2	3	4	5	6	7	8	9	10	Average		
		gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	days
1% pyridine citrate	1	112	8	6	7	8	8	8	8	8	7	8	7	6	15
	2	133	8	7	7	7	8	9	9	9	11	10	8	5	13
	3	116	8	7	9	10	9	10	9	*			8	9	7
	4	235	10	3	8	12	14	12	12	14	13	*	10	9	9
	5	101	7	7	9	7	10	12	12	12	14	11	10	1	13
	6	81	4	7	6	5	8	8	5*				6	0	6
		(av 130)											(av 8 7)		(av 11)
1% pyridine citrate, 1% DL-methio- nine	7†	91	8	0	0	0	8	6	9	9	3	*	4	8	9
	8	143	8	9	9	10	11	11	12	14	14	14	11	3	14
	9	139	8	11	9	12	13	11	8	15	12	7	10	6	120+
	10	245	12	6	7	13	12	12	12	17	16	13	12	0	25
	11	98	2	10	9	6	9	9	9	10	2	7	7	3	34
	12	81	8	6	15	4	6	6	8	8	7	8	7	5	120+
		(av 133)											(av 8 9)		(av 54+)

\* Animal died

† A refusal to eat the experimental diet, such as this animal exhibited, was infrequently observed

The data in this table were obtained from the experiment summarized in chart 3

The animals of this experiment were less uniform in size than those of other experiments

As in the previous studies, the chronic hepatic lesions produced by pyridine in diet 1, and to a lesser extent by pyridine in diet 2, sometimes exhibited advanced stages of fatty infiltration with an early development of extensive diffuse fibrosis The lesions which developed with diets 3 and 4 containing pyridine usually showed little visible fat and consisted essentially of central necrosis together with vascular congestion and hemorrhages, followed by diffuse post-necrotic scarring and finely nodular regeneration

As previously noted, some of the animals on the pyridine-containing diets were observed to survive for weeks or even months with little evidence of illness, only to develop then a sudden and extensive liver necrosis with no apparent additional precipitating cause. The livers of these animals exhibited certain changes prior to the development of the widespread necrosis which will be described elsewhere.

In many experiments employing *ad libitum* feeding, it appeared that animals with the smallest intake of the pyridine-containing diets were the ones which were most likely to develop early fatal liver and kidney damage. On the other hand, some animals were observed to die following a relatively large food intake continuing up to the time of death, and others survived for long periods in spite of continuously or intermittently low levels of food consumption.

*Modification of the effects of the basic diets containing pyridine citrate by supplements of various dietary factors*

The results obtained with *ad libitum* feeding of the four basic diets containing pyridine citrate plus various dietary supplements, are summarized in charts 1, 2, 3, and 4.<sup>5</sup> In general the frequency and degree

<sup>5</sup> As a means of testing certain ideas concerning possible mechanisms of the injury produced by pyridine, the following supplements in addition to methionine, cystine, and choline, have been tried in preliminary experiments, with *ad libitum* feeding in diet 3, for protective effect against pyridine: sodium thioglycollate, British anti-Lewisite (BAL), BAL plus choline, methyl pyridinium chloride, nicotinic acid, yeast nucleic acid, succinylsulfathiazole, pyribenzamine, rutin, thiouracil, thiouracil plus desiccated thyroid, desoxycorticosterone (by daily injection), stilbestrol, continuous inhalation of 100% oxygen, and inhalation of 10% oxygen in nitrogen. The results with thiouracil are shown in charts 1, 2, and 3. Pyridine injury of lethal degree apparently was prevented to a considerable extent by this substance—a point of resemblance of the pyridine injury to the liver injury produced by low protein diets deficient in choline (Gyorgy and Goldblatt, '45). The animals receiving thiouracil developed greatly enlarged thyroid glands, the protection was at least partially abolished by simultaneous administration of desiccated thyroid. The supplements of thiouracil caused a moderate decrease in daily consumption of the pyridine-containing diets. The animals not infrequently appeared ill, and some surviving animals were found to have scarred livers at autopsy. BAL plus choline, breathing 100 per cent oxygen during the experimental period, and particularly large amounts of stilbestrol which greatly reduced the daily intake of the diet, appeared in single experiments with groups containing 6 to 8



of injury observed in surviving animals at autopsy appeared greatest in the groups with the greatest mortality during the experimental periods. However, it was not determined with certainty whether the supplements which afforded protection exerted their effects against the development of all stages of the injury, or only against the late changes which resulted in death. The effects of the individual supplements on growth and on certain morphological aspects of the lesions varied somewhat with the different basic diets (Baxter, '47b), but as judged on the basis of survival of the animals, *the effect of each supplement was strikingly similar with all four of the basic diets*.

*Methionine* When supplements of DL-methionine were added to the pyridine-containing diets, the survival rate of the animals was significantly increased throughout the experimental periods, apparently due to prevention or reduction of liver and kidney injury.

With the various basic diets containing pyridine, the effects of supplements of methionine on food consumption, growth rate, and gain in weight per gram of food were approximately the same as has already been described above in the cases of addition of methionine to the basic diets without pyridine. Food intake and growth rate were considerably increased on the diets of lower protein levels (see growth curves, Baxter, '47b), but with diets 3 and 4, there was usually no significant change in average food consumption, growth rate, or gain in weight per gram of food (tables 3 and 4), and individual animals of the groups with and without methionine could usually be paired fairly satisfactorily on the basis of approximately equal food consumption. In some experiments employing these latter diets, the average food intake of surviving animals was less in those groups receiving methionine than in comparable groups without the supplement. On closer analysis, however, it was usually apparent in those cases that most of the animals which died were those with the smaller intake of food, and the greater survival of animals in the groups receiving methionine, including those with low levels of food consumption, was responsible for the lower average food intake in these groups (table 3).

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animals each, to decrease the liver damage and mortality. These supplements, together with tocopherol, probably deserve further study. The remaining substances had no apparent effect on the results, with the possible exception of breathing 10 per cent oxygen, and daily desoxycorticosterone injections, which seemed to increase damage.

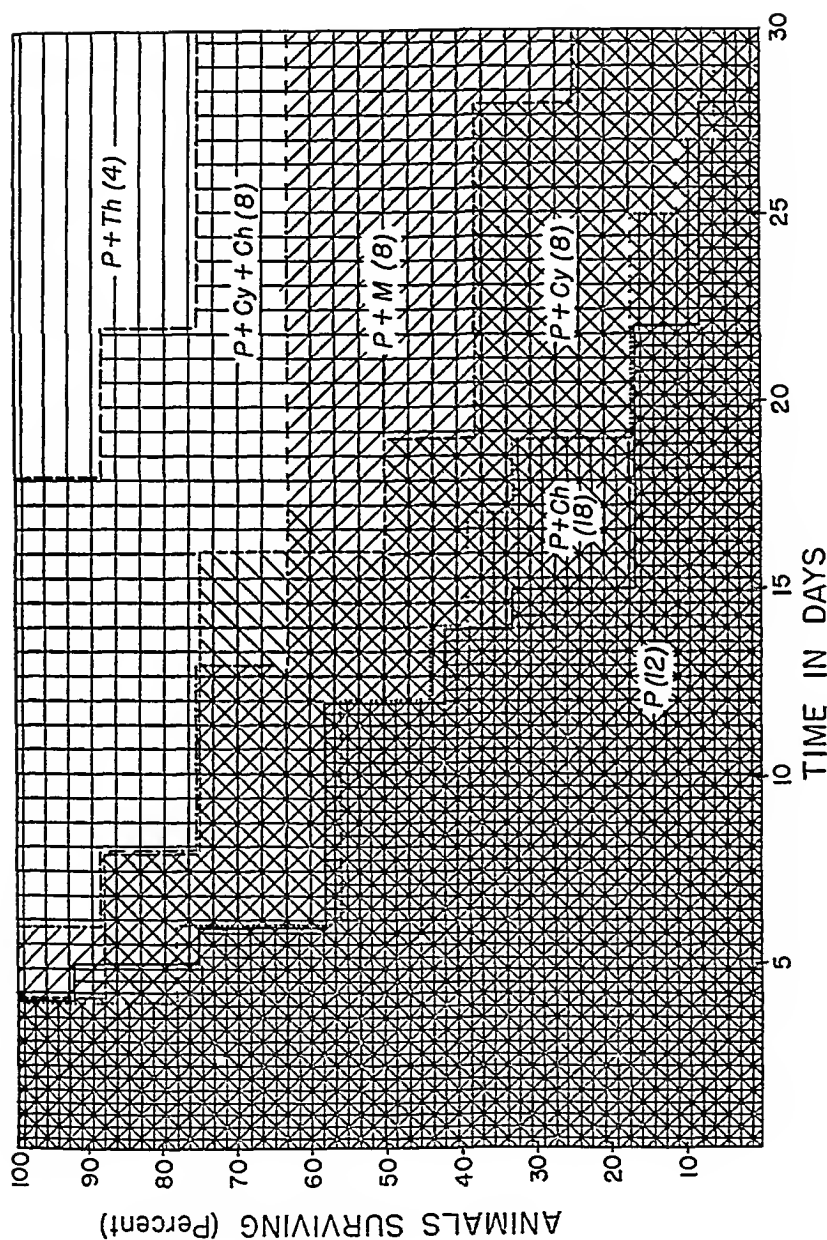


CHART 1 Results with *ad libitum* feeding of diet 1 containing pyridine citrate alone and in combination with the various supplements, the concentrations of which are indicated in table 2. The numerals in parenthesis indicate the number of animals in each group.

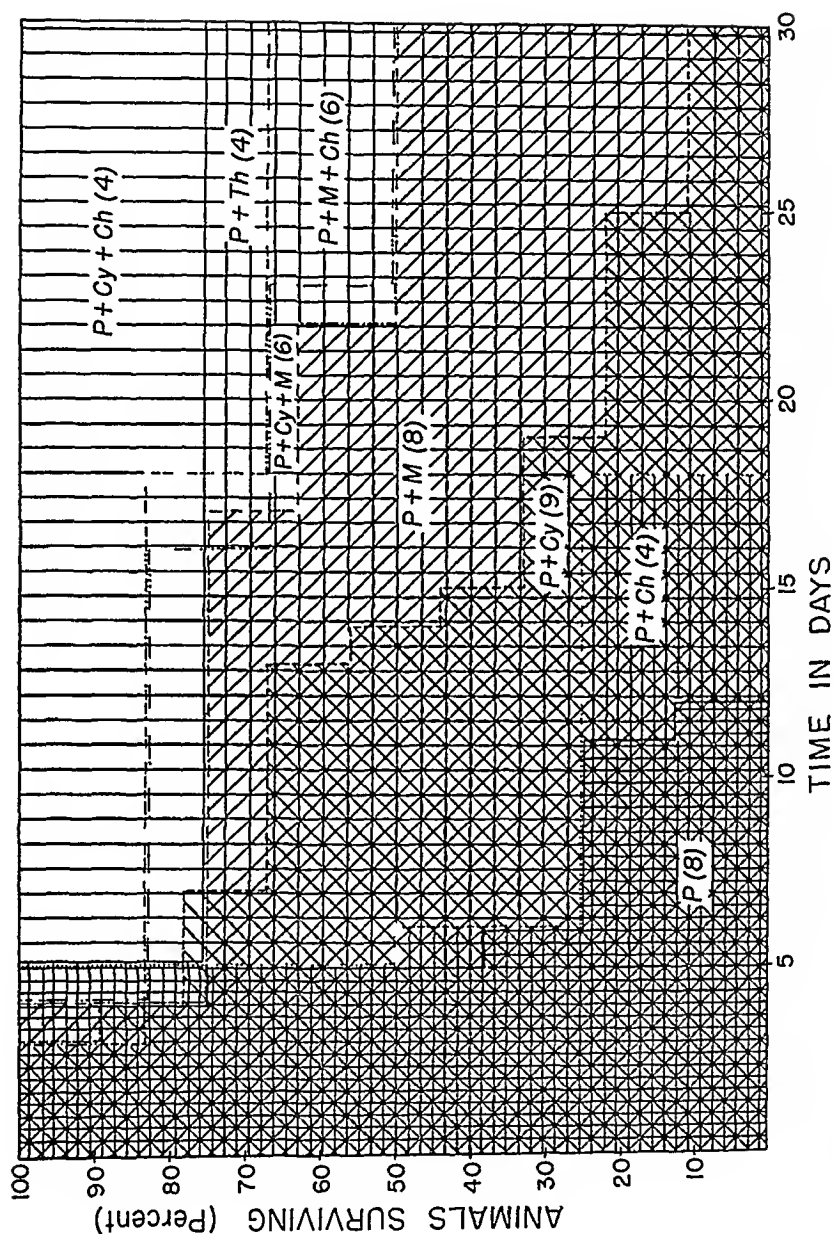


CHART 2 Results with *ad libitum* feeding of modifications of diet 2. For explanation, see table 2 and the legend of chart 1. In the combination P + Cy + M, 0.5% methionine instead of 1.0% methionine was used. P + Cy + M and P + M + Ch were used only with diet 2; the results with these combinations are indicated in the chart by graph lines alone, with no hatching beneath

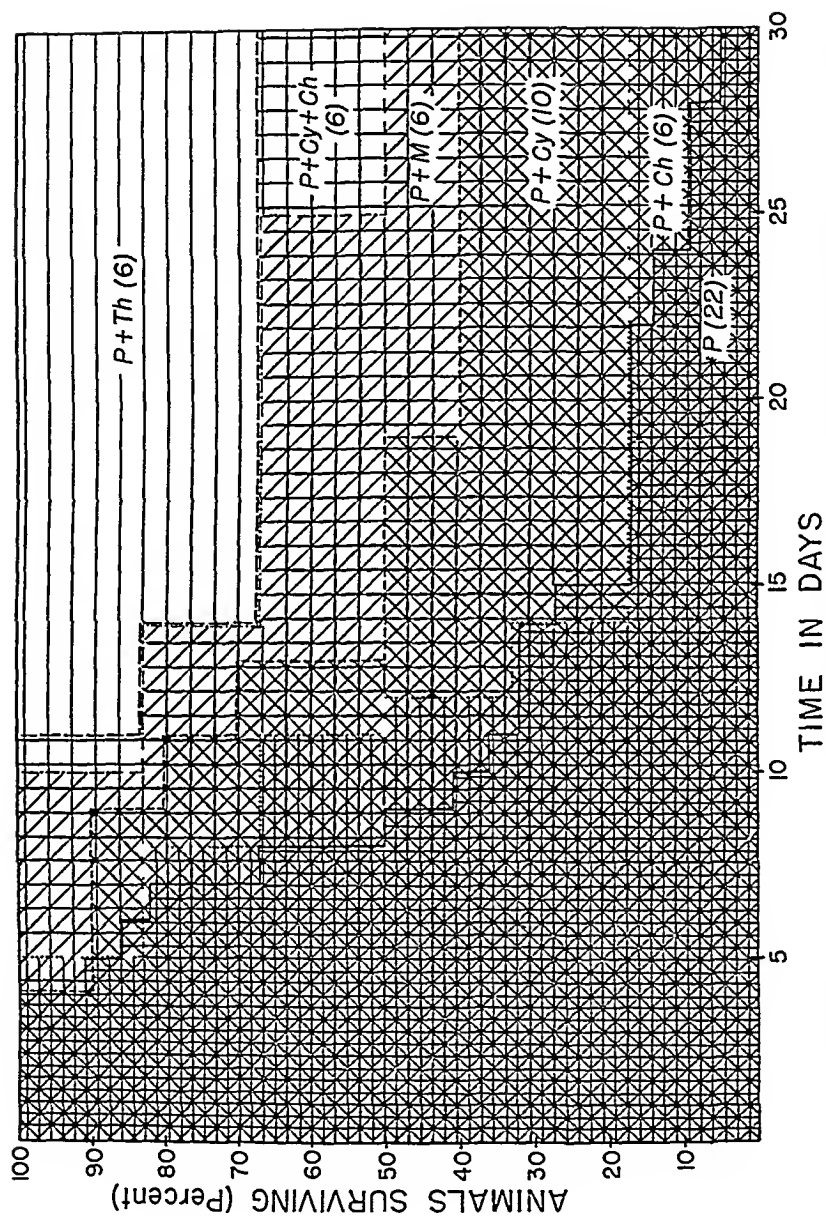


CHART 3 Results with *ad libitum* feeding of modifications of diet 3 See table 2 and legend of chart 1 for explanation

*Cystine* Supplements of L-cystine, in amounts equivalent on the basis of sulfur content to the DL-methionine used, were employed with the first three diets. In general, the animals receiving this supplement grew at about the same rate as those receiving methionine. Cystine caused some increase in survival of the animals, but it was

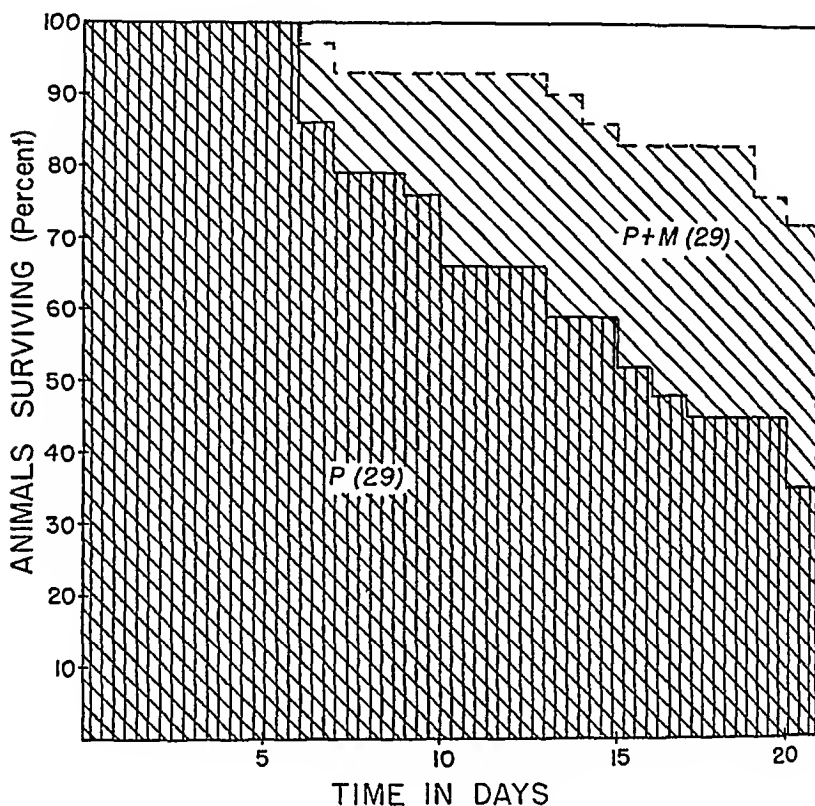


CHART 4 Results with *ad libitum* feeding of modifications of diet 4. For explanation, see table 2 and legend of chart 1.

not as effective as methionine. Twice the equivalent amounts of cystine in one experiment did not improve the results. Particularly on the first two diets, the livers of the animals receiving cystine appeared to be larger and more fatty than those of most of the animals receiving pyridine alone, but necrosis in addition to fatty changes was observed in the livers of the cystine-treated animals which died.

*Choline* Choline chloride in amounts equivalent, on the basis of content of methyl groups, to from 1 to about 6 times the amount of methionine employed, were added to the pyridine-containing diets. On none of these choline-supplemented diets did the animals significantly outlive the controls. Food consumption was uninfluenced or slightly decreased by supplements of choline. In spite of the failure of choline to decrease mortality, choline apparently did produce some modification of the lesions which occurred with diets 1 and 2, including a reduction in the fatty changes, and it is possible that some decrease in mortality attributable to choline might have been observed in experiments of longer duration, provided that a sufficient number of the pyridine-treated animals had survived the early periods.

*Cystine plus choline* In the experiments with diets 1 and 2, the groups receiving the combination of cystine and choline appeared healthier than those of any other groups, and survival of the animals in the groups receiving the combination was considerably greater than that with cystine or choline alone, and at least as great as with methionine<sup>6</sup>. Not only was fatty infiltration of the liver less with cystine plus choline than with cystine alone, but necrosis also was less when choline was added simultaneously. Even on diet 3, it appeared that the protective effect of cystine plus choline, like that of methionine, was probably greater than that of cystine alone.

#### RESULTS WITH PAIRED FEEDING

Although it appeared from the results of the experiments with *ad libitum* feeding that the protective effect of methionine supplements was not due to a modification of the level of food and pyridine consumption by this supplement, never-the-less the effects of additions of methionine were further investigated in paired-feeding experiments conducted as already described. The results of the latest and most satisfactory of these experiments are shown in chart 5. The animals of the groups allowed 3.2 grams of food daily invariably ate all of the diet offered, and consequently the daily food intake of all of the animals of these groups was equal.

<sup>6</sup> It should be noted that while the cystine of this combination was equivalent, on the basis of sulfur content, to the methionine used in each experiment, the choline of the combination was considerably greater than the methionine in its content of methyl groups.

*Effects of limitation of intake of pyridine-containing diets*

In one small group of animals fed 6.4 grams daily of diet 4 containing pyridine (chart 5), it appeared that the average length of survival was appreciably greater than in a similar group of animals allowed only 3.2 grams of the diet per day. This observation suggested that the beneficial effects of the greater food intake more than outweighed any deleterious effects of the increased simultaneous intake of pyridine. The animals consuming 3.2 grams of food per day grew much less rapidly than those receiving 6.4 grams, but they continued to gain weight slowly and appeared to be adequately nourished throughout the experimental period. The animals receiving 6.4 grams of food grew rapidly, they usually ate all of the allotted food but occasionally left small amounts unconsumed. The level of food intake was greater than observed in certain groups of animals of similar size with *ad libitum* feeding.

It has already been pointed out that the animals with the lowest levels of daily food intake in the *ad libitum* feeding experiments were often the first to become ill and die. Whether the low level of food consumption was a cause or a result of injury could not always be determined. On the other hand, certain substances which moderately (thiouracil) or considerably (stilbestrol) decreased daily consumption of the pyridine-containing diets, appeared in preliminary experiments to afford protection against the diets. Perhaps these substances afforded protection in spite of, rather than because of, the decreased food consumption, but further studies are necessary.

*Effects of methionine supplements*

In all of the paired-feeding experiments, as in the experiments with *ad libitum* feeding, survival was greater throughout the experimental periods in groups receiving supplements of methionine than in the corresponding groups not receiving these supplements. This protective effect of methionine is well illustrated by the results shown in chart 5. The growth rate of animals receiving 3.2 grams of the pyridine-containing diet with and without methionine, was approximately equal. All of the surviving animals of the group receiving supplements of methionine appeared in moderately good condition at the end of the three-weeks experimental period.

*Effects of omitting pyridine from the diet*

At the end of three weeks, six of the eleven surviving animals of the group receiving the pyridine-containing diet with added methio-

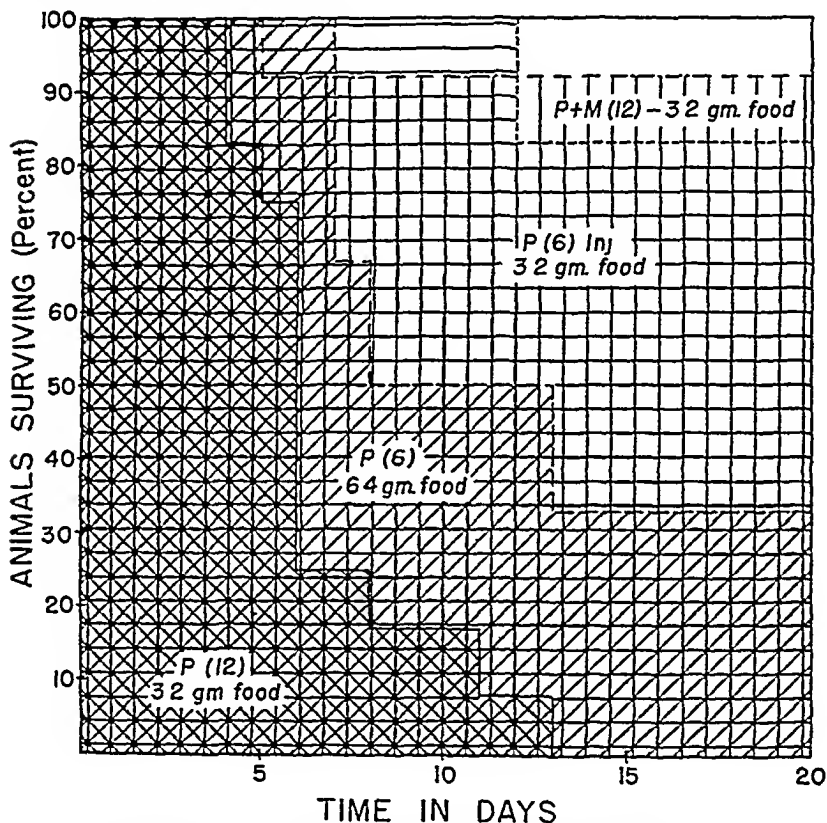


CHART 5 Results with *controlled* feeding of diet 4, comparing effects of two different levels of daily intake of the pyridine-containing diet, the effects of pyridine in the diet and pyridine by injection, and the effects of the pyridine containing diet with and without added methionine. See table 2 and legend of chart 1 for explanation of symbols

nine (chart 5) were changed to 3.2 grams daily of diet 4 from which the pyridine citrate had been omitted. The remaining five animals were continued on the pyridine-containing diet. Within 24 hours, two of the six animals changed to the diet without pyridine were dead, while



all of the animals continued on pyridine survived and appeared well. Pyridine was later omitted from the diet of the five animals continued on pyridine, and one of them died within the following 24 hours. This observation would have been considered a coincidence of no significance except for the fact that by way of terminating experiments on two occasions in the past, the animals surviving after several weeks on pyridine-containing diets were placed together in cages with stock diet of which they ate hardily, and a considerable proportion of them were found dead the next morning.

### *Effects of injected pyridine*

The small group of animals receiving pyridine by injection (chart 5) were given daily 0.65 cc of a 2 per cent solution of pyridine base (approximately 30 per cent more pyridine than was eaten in the diet by the animals receiving an equal amount of diet containing pyridine citrate) subcutaneously for one week, 0.65 cc intraperitoneally during the second week, and 1.2 cc intraperitoneally during the third week. The injections always were given 30 minutes after the animals received the daily diet allotment. Particularly during the latter part of the experimental period, those animals receiving pyridine by injection appeared ill and did not eat all of the limited amount of food offered them. However, survival of the animals was much greater in this group than in the group receiving pyridine citrate in the diet. There was little or no evidence of liver or kidney injury in the animals receiving injections, and it was doubtful that such injury caused the death of the single animal which did not survive.

### DISCUSSION

When the sulfur-containing amino acids were employed in the early experiments with diet 1, definite protective effects were observed. However, it seemed important to define further the circumstances under which these supplements might be expected to afford protection against pyridine, and to determine if possible the mechanism of the effect. As with diet 3 in the previous study (Baxter, '47b), results with diet 4 indicated that the protective effects of methionine and cystine were not due entirely to increasing the nutritional value of the diet (as measured by gain in weight per gram of food in rats under normal

conditions) by raising the levels of essential factors previously present in the diet in suboptimal amounts. With the earlier diets, on which supplements of methionine or cystine caused an increase in food and pyridine consumption, it is possible that part of the protective action of the supplements was due to a beneficial effect derived from the increased food intake which more than balanced the harmful effect of the simultaneously increased consumption of pyridine. With diets 3 and 4, however, it did not appear that the protective effect of the supplements could be explained on the basis of any modification of the food or pyridine consumption, and this impression was confirmed by the results of the paired-feeding experiments.

Choline (Best and Huntsman, '35) and methionine (Tucker and Eckstein, '37) have been shown to have a lipotropic effect on fatty livers produced by dietary means, and to prevent cirrhosis produced in a similar manner (Blumberg and McCollum, '41, Daft, Sebrell and Lilhe, '41, Gyorgy and Goldblatt, '42). Most studies indicate that it is the choline molecule (Welch, '36, Moyer and du Vigneaud, '42), probably through its role in the transport of fat from the liver to fat depots (Stetten and Salcedo, '44), that is lipotropic, and that methionine is active in this respect only through its ability to donate methyl groups for the formation of choline in the body (du Vigneaud, Chandler, Moyer and Keppel, '39, du Vigneaud, Cohn, Chandler, Schenck and Simmonds, '41). Cystine, on the other hand, has generally been recognized to have an antilipotropic effect (Curtis and Newburgh, '27, Beeston and Channon, '36, Tucker and Eckstein, '37, Gyorgy and Goldblatt, '42, Earle and Victor, '42), probably by increasing the synthesis of liver fat (Stetten and Salcedo, '44).

Not only fatty infiltration and subsequent fibrosis, but also liver necrosis and hemorrhages, have been observed to follow the administration of diets deficient in both choline and the sulfur-containing amino acids, and several investigators have obtained results which strongly suggested that these lesions were not all a part of the same pathological process, nor all the result of the same single (choline) deficiency (Gyorgy and Goldblatt, '42, Earle and Victor, '42, Daft, Sebrell and Lilhe, '42). This subject has recently been extensively investigated by Himsworth and associates (Glynn and Himsworth, '44, Himsworth and Glynn, '45a, '45b), who have extended these

earlier observations with the conclusion that dietary fatty infiltration and cirrhosis (diffuse portal fibrosis) are due to deficiencies of lipotropic substances (choline and precursors), while dietary hepatic necrosis and sequelae are due to deficiencies of sulfur-containing amino acids. By using amino acid mixtures containing various levels of methionine and cystine, in place of dietary protein, it was observed that while methionine was necessary for growth and maintenance of normal plasma protein and erythrocyte levels, hepatic necrosis was prevented by cystine in the absence of methionine (Glynn, Himsworth and Neuberger, '45). Methionine, and cystine plus choline (Gyorgy and Goldblatt, '42, Daft, Sebrell and Lillie, '42, Green and Brunschwig, '46), as might be expected, have been found effective against both fatty changes with subsequent fibrosis, and also necrosis, produced by dietary means.

The fact that fatty infiltration which was often observed in the present studies when pyridine was added to diet 1, and to a lesser extent with diet 2, was largely eliminated by the use of diets 3 and 4, which contained optimal levels of choline and sulfur-containing amino acids, suggested that the fatty changes were chiefly due to dietary deficiencies, which were made more evident by the addition of pyridine but not actually caused by pyridine itself, though this conclusion could not be arrived at with certainty.<sup>7</sup> It seemed likely, as previously pointed out (Baxter, '48a), that necrosis was the important parenchymal lesion attributable to pyridine *per se*, and that the cirrhotic lesions which developed in the later periods on diets 3 and 4, despite the finely nodular appearance and the uniform distribution of the fibrotic areas,

<sup>7</sup> The results of unpublished studies done in collaboration with Dr C. T. Ashworth, showed that the fatty changes produced by diet 1 containing pyridine were decreased but not prevented by choline alone. They were more completely prevented (as was the necrosis which occurred at the same time) by the addition of choline plus cystine, or methionine, while they were often intensified by cystine alone. Interpretation of these observations was complicated by the fact that food intake on diet 1, after the addition of pyridine, was small, and was substantially increased by the addition of sulfur-containing amino acids or casein, but not by choline alone (Baxter, '47b). The observations seemed to indicate that the fatty infiltration was not due entirely to a deficiency of choline, but that it was perhaps due in part to the decrease in food intake which often accompanied the addition of pyridine to the diet. Best ('34) observed that under certain conditions the fatty infiltration of starvation was not prevented by choline.

were a result of necrosis with persistence of unresolved necrotic areas, and thus were different in pathogenesis from the cirrhosis with periportal fibrosis usually occurring in fatty livers. Fatty changes sometimes did occur, however, particularly in the early lesions, even with diets 3 and 4. Concerning the question of the distinctiveness of the fundamental disturbances which result in fatty infiltration, on the one hand, and necrosis, on the other, the results allowed no definite conclusions. It is perhaps significant that the sulfur-containing amino acids, but not choline, afforded definite protection against hepatic necrosis, both as it occurred in the present and other (Miller, Ross and Whipple, '40) experiments with toxic agents, and also in the studies discussed above employing deficient diets, though the degree of protection of course differed greatly under the two sets of circumstances. The possible effects of varying the amounts of tocopherol in the diets (Gyorgy and Goldblatt, '49) have not been studied specifically, though diets 3 and 4 contained ordinarily adequate amounts of this factor, while none was added to the earlier diets.

The enhancement by choline of the protective effect of cystine, in spite of the ineffectiveness of choline alone, such as was observed in the present study, has been noted by others under other circumstances (Gyorgy and Goldblatt, '42, Glynn, Himsworth and Neuberger, '45, Green and Brunschwig, '46), but the explanation of this phenomenon has not been entirely clear. Since methionine is a dietary essential which cannot be replaced by cystine (White and Beach, '37), though it can be replaced by the unnatural amino acid, homocystine, when accompanied by an adequate supply of methyl donors (du Vigneaud, Chandler, Moyer and Keppel, '39), there seems to be little evidence upon which to postulate that the effectiveness of the cystine-choline combination was due to the ability of the body to form methionine from this combination. Furthermore, the effectiveness of the combination under the conditions of the present experiments did not appear to be due in whole to a sparing of dietary methionine, but rather to actions of the supplements *per se*.

One possible explanation of the greater effectiveness of the cystine-choline combination or of methionine, than of cystine alone, is that two independent pathological processes—perhaps necrosis and fatty infiltration—resulted from the administration of pyridine, one of

which was counteracted by cystine and the other by choline, while methionine was effective against both as a result of its dual role in the body. An explanation more completely in keeping with the present observations appears to be as follows. Cystine or a related substance was the factor which afforded protection against the necrosis produced by pyridine. However, when the diet was already deficient, or adequate but limited, in choline, cystine produced in addition to its protective action, toxic effects of its own due to the induction of a relative choline deficiency, which limited the beneficial effect of cystine, particularly in the later periods. When choline was added at the same time as cystine, the toxic actions of cystine (in the amounts employed) were neutralized by the choline, and the protection against pyridine was at least as great as with methionine.

That the effects of pyridine-containing diets were not determined alone by the pyridine dosage, but were influenced to a substantial degree by certain other dietary and metabolic factors, was indicated not only by the protective effect of the sulfur-containing amino acids against injury produced, but also by several additional observations. In the first place, the effects of pyridine which have been described, were regularly produced only when pyridine was fed in the diet, pyridine appeared to be less toxic when administered separately, according to the schedule employed. Furthermore some evidence seemed to indicate that limitation of the daily intake of the toxic pyridine-containing diets, at least within certain ranges, increased rather than decreased the injury and mortality resulting from the diets. The results with thiouracil supplied further evidence that the injury was influenced by general metabolic factors. Finally, observations on several occasions suggested that a significant rate of mortality might have been associated with the omission of pyridine from the diet of animals after they had survived for several weeks on pyridine-containing diets.

The observation that the addition of 2 per cent succinylsulfathiazole did not influence the effects of the pyridine-containing diets (footnote 5), constituted some evidence against the supposition that the greater toxicity of pyridine when incorporated in the diet might have been due to formation of a more toxic substance from pyridine by bacterial action, and the presence of the odor of pyridine in the tissues of pyridine-injected animals for more than 24 hours after the last injection.

cast doubt on the possible explanation of this phenomenon which was previously suggested, that the greater toxicity of pyridine when ingested in the diet was due to its more nearly continuous presence in the body when administered by this method

The wide variation in the effects of pyridine-containing diets noted in various individual animals, and the observation that some animals on the pyridine-containing diets developed extensive hepatic necrosis without obvious explanation after ingesting the diets for long periods of time without significant illness, prompted consideration of the possibility that the effects of pyridine might be to a considerable degree conditioned or complemented by other environmental factors, such as, for example, intercurrent infectious diseases. Although such a possibility could not be entirely eliminated, it does not affect the significance of the results

Sufficient data has not been obtained on the effects of different concentrations of pyridine in a single diet, with intake of the diet maintained constant, to draw definite conclusions regarding the degree of correlation which exists between pyridine dosage and toxic effects, when other variables are eliminated

The results of the experiments illustrated well that the physiological effects of administered substances may be influenced by composition and quantity of the diet, and that the effects of single dietary factors may be influenced by the levels of certain other dietary factors. Comparison of racemic methionine with the natural cystine isomer was undesirable, but perhaps permissible (Jackson and Block, '38)

Since it would have been necessary in any event to measure and control food intake, no obvious disadvantages resulted from the necessity of administering the hepatotoxic substance in the diet. The long-term experiments were facilitated by the ease of administration of the substance in the diet, and it was perhaps possible by the continuous administration of the injurious agent, to reproduce more closely conditions responsible for certain types of clinical liver injury than can be accomplished by the injection of hepatotoxic substances at infrequent intervals. Finally, it should be pointed out that the increase in survival produced by supplements of the sulfur-containing amino acids probably should be regarded as due chiefly to prevention of injury, or of certain complications of the injury, rather than to cure of injury

## SUMMARY

Pyridine, when administered to rats in diets containing low levels of *casein* and *choline*, appeared to induce both necrosis and fatty changes in the liver, but with more nearly optimum diets, necrosis along with vascular engorgement and hemorrhages in the central areas, and their sequelae, were the prominent morphological alterations which resulted. Renal damage often occurred in animals with hepatic injury.

The injury induced by *ad libitum* or *controlled* feeding of the pyridine-containing diets was prevented to a considerable extent by supplements of *methionine*, and survival of the animals was significantly increased. *Cystine* also produced some degree of protection, but with the basal diets employed, none of which contained greatly excessive quantities of *choline*, *cystine* was less effective than *methionine*. *Choline* alone, did not appreciably influence survival of the animals, however, it did enhance decidedly the protective effect of *cystine*. The protective effect of *cystine plus choline* appeared greater than the sum of the effects of the individual factors, and this combination was at least as effective as *methionine*.

Observations on small groups of animals with *ad libitum* feeding, suggested that a considerable degree of protection was provided against fatal pyridine-induced injury by thiouracil, perhaps as a result of its general metabolic effects.

The protection afforded by the sulfur-containing amino acids, at least in the diets already containing the higher levels of these factors, did not appear to be the result of an increase in the nutritional value of the diets as measured by gain in weight per gram of food under normal conditions, or of any influence on intake of food and pyridine. The mechanism of the protection afforded by methionine against the toxic effects of pyridine in the diet was not determined, nor is it possible to predict the extent of the circumstances under which a similar protective effect of methionine against liver and kidney injury might be expected to occur.

The effects of the individual dietary factors are considered in relation to their known nutritional and metabolic actions, and possible explanations of the greater effectiveness, under certain conditions, of the cystine-choline combination than of cystine alone are discussed.

Pyridine was observed again to be more toxic when administered in

the diet than when given by daily parenteral injections. Limitation of intake of pyridine-containing diets perhaps increased rather than decreased the toxic effects produced, and a possibly significant rate of mortality was observed when pyridine was omitted from the diet of rats which had survived for several weeks on pyridine-containing diets.

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## PLATE 1

FIG 1 A section from the liver of a rat which became critically ill with pale, cold skin after 2 weeks on diet 3 containing pyridine, and was autopsied at that time. The liver was enlarged, dark, and engorged with blood. The section shows extensive necrosis in the central portions of the lobules, with many red blood cells in the necrotic areas. The walls of many of the sinusoids and central veins within the necrotic areas showed evidence of severe damage, with extravasations of blood. The hepatic cells about the vessels of the portal triads (sectioned obliquely) appeared normal. Hematoxylin and eosin stain  $\times 40$ .

FIG 2 Section from a kidney of a rat after 1 month on diet 1 containing pyridine. The animal did not appear definitely ill when autopsied, but recently had survived a severe episode of illness and was not eating well. The liver appeared finely nodular, sections showed thin bands of fibrous tissue and marked fatty infiltration of parenchymal cells. The section of kidney here illustrated shows degenerative changes in the cells of the convoluted tubules, with many dilated tubules lined by flattened epithelium and containing some protein material. The dilated tubules extended well down into the medulla. Scattered foci of calcification, and of active cellular proliferation, were present. This type of injury was not prevented by choline. Hematoxylin and eosin stain  $\times 110$ .





MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY  
IN ASSOCIATION WITH THE ALPHA OMEGA  
ALPHA FRATERNITY<sup>1</sup>

HURD HALL, THE JOHNS HOPKINS HOSPITAL  
MONDAY, APRIL 11, 1949

Dr Harvey The first paper on the program tonight is "The Effect of Urethane on the susceptibility of Mice to Pneumonia Virus of Mice" by Mr Charles Ira Leftwich, Jr Discussion by Dr George S Mirick

*The Effect of Urethane on the Susceptibility of Mice to Pneumonia Virus of Mice*  
(PVM) CHARLES I LEFTWICH, JR

Pneumonia virus of mice (PVM), first described by Horsfall and Hahn in 1940, is a small strictly pneumotropic virus infectious for mice and, after adaptation, certain other rodents When inoculated intranasally in sufficient amount, mice die with an interstitial pneumonitis The virus will under certain conditions agglutinate mouse erythrocytes

Nettleship and Henshaw in 1943 reported that urethane had the capacity to stimulate the development of pulmonary tumors in susceptible strains of mice It seemed of interest to study the effect of urethane on PVM It was found that the administration of urethane orally or intraperitoneally, either before or immediately after PVM inoculation, resulted in a more extensive pneumonitis than in control mice The extent of the pneumonitis was related to the urethane dosage, and amounts of urethane which produced the effect on PVM did not in themselves produce pneumonitis Not only was the pneumonitis more extensive, but the amount of PVM in the lungs of mice receiving urethane was greater than in controls when titrated either by hemagglutination or for infectivity The capacity of mice to develop antibodies when immunized with PVM was not impaired by urethane The mechanism by which urethane, which is reported to block cell division, can result in increased virus multiplication has not been elucidated

Dr G S Mirick I have been very interested in the work which has been reported by Mr Leftwich

The observations, that the susceptibility of a laboratory animal to a virus infection may be increased as the result of treatment with a drug, suggests this as a possible approach to the isolation of certain viruses One wonders whether other viruses, particularly viruses affecting other systems of the host, would be similarly

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<sup>1</sup> This meeting, the final meeting of the Johns Hopkins Medical Society held in association with the Alpha Omega Alpha Fraternity, emphasized the contributions, which were made essentially by students of the School of Medicine

affected by this drug The demonstration that urethane, which stimulates the development of pulmonary tumors in mice also stimulates a virus which is normally latent in the lungs of certain strains of mice, raises the question of the possible relationship between the virus and this neoplasm

*Dr Harvey* The next paper on the program is "Use of Bal-Mapharsen Compound in Mouse Trypanosomiasis" by Messrs Benjamin Burrows, John L. Sawyers and Thomas H. Maren Discussion by Dr Morris Rosenthal (The original data upon which this presentation was based was published in the Proceedings of the Society for Experimental Biology and Medicine, 70 194-197, 1949)

*Dr Rosenfeld* We were disappointed that the condensation of BAL with Oxophenarsine resulted in a product with about one-third the therapeutic index of the parent arsenical The therapeutic index, however, is a quantity that is sensitive to the procedure used in testing therapeutic and toxic action and a lesser discrepancy might appear under different conditions of measurement

An approach similar to that of these workers has led to the development of a new diuretic agent which seems to give promise of clinical usefulness This drug, thiomerin, is a mercaptide of sodium mercapto-acetate and the organic mercurial present in mercuriophylline In this case, acute cardiac toxicity was very much reduced although the more chronic toxicity, as measured by the 4 day lethal dose, was not appreciably changed by formation of the complex A particularly favorable attribute of the mercuriol mercaptide is the diminished local irritation and tissue injury I wonder whether the BAL-arsenical condensation product would be sufficiently devoid of local necrotizing action to permit intramuscular use?

*Dr Harvey* The next paper on the program is "Objective Testing of Vision with the Use of Galvanic Skin Response" by Mr Henry N. Wagner, Jr Discussion by Dr John E. Bordley

*Objective Testing of Vision with the Use of the Galvanic Skin-Resistance Response*  
HENRY N. WAGNER, JR., Psychobiological Laboratory, Phipps Clinic, Johns Hopkins Hospital

Our aim is to outline a simple technique for the objective testing of vision We have found a technique that is useful as an aid in the diagnosis of psychogenic or malingering factors in visual defects in man, whether the defects are present as a failure of visual acuity or as a limitation of the visual field, and that, with modifications, can be used for the study of vision in animals

We use a skin galvanometer that gives a graphic record of activity in the sympathetic nervous system, recording from the palm and back of the hand the changes in the electrical resistance that the skin offers to the passage of a minute direct electrical current For visual acuity testing, the patient is first conditioned to expect a mild electric shock when a given letter is flashed on a screen which he faces By projecting the eye chart, one letter at a time, passing the threshold in

both directions, one obtains the visual acuity by observing the wave record of the sympathetic activity. For perimetry, the patient is conditioned to respond to the sight of the white target dot. After the conditioning, the dot is slowly moved into the patient's field from the periphery and the response when it comes into the visual field is obtained. Visual acuity has been studied by this method in four children, ages 5 to 13, in three cases of psychogenic blindness, in five Wilmer Institute patients, and in seven normals. Objective perimetry studies have been made in only one person.

In the case of animals, to produce the immobility and fixed gaze necessary for visual studies with the galvanic skin response, we make use of a drug, bulbo-capnine, which produces a cataleptic state in animals, sometimes referred to as experimental catatonia. Although immobilized, the animals (monkey, cat, and rat in our experiments) give active galvanic skin resistance responses to sensory stimuli of all types.

*Dr. Bordley:* The work that Mr. Wagner has presented tonight and spoken so briefly of is not only of great interest but is the result of a lot of hard labor. Any one who has worked with Dr. Richter's skin resistance apparatus can appreciate the difficulties in obtaining the excellent results Mr. Wagner has shown us. My remark about Dr. Richter's skin resistance technique does not mean that we have not found it of the greatest service in the work that Dr. Hardy and I have been doing in audiometry. We think this method offers a quick and accurate determination for certain hearing problems, but the control of children is a very time-consuming and difficult job. The work that Mr. Wagner spoke of that has been done with bulbo-capnine is of particular interest to people working with conditioning of children or animals, and I believe when this approach has been further explored, we may find that he has made a real contribution in the development of a new technique to be employed in studying conditioned reflexes. I think Mr. Wagner should be congratulated.

*Dr. Harry:* The next paper on the program is "Biochemical and Morphological Differentiation in the Developing Cerebral Cortex" by Mr. Elston L. Belknap, Jr. Discussion by Dr. Louis B. Flexner.

*Biochemical and Morphological Differentiation in the Developing Cerebral Cortex*

ELSTON L. BELKNAP, JR. (Department of Embryology, Carnegie Institution of Washington, Henry Strong Dennison Scholar in Embryology, 1948-49)  
(Introduced by Dr. Louis B. Flexner)

Evidence presented herein shows that there is a critical period in the cytological, biochemical, and possibly functional differentiation of the cerebral cortex of the fetal guinea pig. This period is between the 41st and 45th days, or two-thirds of the way through gestation. Striking changes have been found by the group at the Department of Embryology of the Carnegie Institution. During this period, Nucleoside substance suddenly appears. The nucleus reaches its final volume at about the



same time Cell processes suddenly start to sprout in great numbers, and there is a change in their refractive index to that characteristic of the adult During this same time interval there is a striking rise in apyrase activity, an enzyme which releases stored energy Immediately following this period, at the 45th day, spontaneous potentials can be recorded from the cortex for the first time, with the reservation that it is not proved at present that these potentials arise in the cortex

My part in the program consisted in the study of certain respiratory enzymes Just after the four day period of morphological differentiation succinic dehydrogenase activity rises abruptly to reach the adult level Another respiratory enzyme, cytochrome oxidase, does not increase in activity until about term

This critical period in differentiation is not peculiar to the guinea pig cerebral cortex It has been found in the cortex of the fetal pig by the Carnegie investigators and in the newborn rat brain by other investigators

It is important to remember that these changes in structure and chemistry can not yet be correlated as to cause and effect At present one can only speculate as to the meaning of the changes

*Dr Flexner* The histochemical methods which Mr Bellnap and others of us have been forced to use in these studies have certain limitations We are primarily interested in changes in the neurons of the cerebral cortex We analyze, however, a sample of cortex containing nerve cells, processes of nerve cells and glia Our interpretations will be on firmer ground when we learn about the biochemistry of glia and then correct our chemical analyses on the cortex for its content of glia

*Dr Harvey* The final paper on the program this evening is "The Histochemical Localization of Cholinesterase Activity" by Mr George B Koelle and Dr Jonas S Friedenwald Discussion by Dr J G Lilienthal, Jr

*The Histochemical Localization of Cholinesterase Activity*<sup>2</sup> GEORGE B KOELLE AND JONAS S FRIEDENWALD, Wilmer Institute, Johns Hopkins University and Hospital

The localization of cholinesterase (ChE) in various tissues has been studied previously by indirect means (1, 2) Gomori (3) has published a histochemical technique for this purpose, but his data suggest that the method localizes non-specific types of ChE and not the physiologically important specific ChE In the present method the substrate employed is acetyl-thiocholine (AThCh)(4), which is hydrolyzed at a greater velocity than acetylcholine by both types of enzyme Frozen tissue sections or teased preparations are incubated for various periods in a solution containing AThCh and copper glycinate, which is buffered to pH 8.06 and saturated with copper thiocholine As the substrate is hydrolyzed to thiocholine by the enzyme present, copper thiocholine is precipitated, which is converted to

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<sup>2</sup> This work was supported by the National Institute of Health, John and Mary Markle Foundation, and Chalfant Fund

copper sulfide by subsequent treatment with ammonium sulfide. The amorphous dark brown deposits of copper sulfide thus indicate the sites of enzymatic activity. In control preparations previously treated with diisopropyl fluorophosphate the characteristic staining does not appear.

In the intercostal muscles of the rat, ChE has been found to be concentrated most heavily in the regions of the motor endplates, as was suggested by earlier studies (1, 5). The ChE in the medulla oblongata of the rat is located within the neurons and the glial cells, where the cell nuclei are most heavily stained. Other sites of ChE activity noted include the ganglion and satellite cells of the sympathetic ganglia (cat), ganglion cells of the ileal mesenteric plexus (rat) and adrenal medulla (cat), and the chromaffin cells of the latter tissue. In the retina of the albino rabbit, ChE activity appears to be confined largely to the layer of bipolar cells.

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*Dr J. G. L. Herlihy, Jr.* This is an exceedingly important fundamental contribution to the field. The elegant technique provides a tool for wide application to many problems hitherto not susceptible to direct analysis.

It is worthwhile recalling that the current, useful schema describing the interaction of acetylcholine and cholinesterase mechanisms, *in situ*, is based almost entirely on indirect evidence. The basis for the plausible concept that cholinesterase is localized in relatively high concentration at junctional sites is not the result of direct proof, with the single exception of the electric organ of the electric eel. The work presented here provides strong, direct support for current hypotheses. And when the method has been developed further to distinguish between various types of cholinesterase, it will provide a superb tool for direct analysis.

The present lack of complete specificity is the probable explanation of the finding of apparently widespread distribution of the esterase in supporting tissue.

There is another aspect of this work which is most encouraging. To echo Dr Herlihy's comments, I would like to stress the difficulties attending an "chemical dissection" of tissues in the isolation of a system dispersed or localized in a complex cellular organization. This technique of Drs Koelle and Frieden has provided the means to circumvent several difficulties involved in precise localization.

I should like to congratulate you on this important contribution.

## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

*Current Therapy*, 1949 Edited by HOWARD F. CONN 1949, 672 pp \$10.00 W. B. Saunders Co., Philadelphia

This book gives in concise outline form the therapy of medical diseases written by selected experts in their fields. It fulfills a need of the practitioner who can quickly obtain a guide to therapy, when therapy has made such rapid changes in the last decade. This type of book assumes that the physician has a good deal of judgment and experience and can apply the therapy with proper regard for the change in symptomatology, physical signs and diagnostic tests in each case. Each brief outline of therapy is the distilled and crystallized experience of the expert from observation of many patients. In applying the therapy, due consideration of the individual case variations is assumed. For this reason, this book is very valuable to the practicing physician with clinical experience and would not be a good text for the teaching of therapy to medical students or inexperienced staff, who need emphasis on the development of skill in observing the patient and development of judgment in each case based on a knowledge of fundamental physiologic and pharmacologic principles.

It is highly commendable that on many subjects, more than one (and sometimes conflicting) opinion is given, thus indicating that no outline of therapy is a rigid gospel, but is a very helpful organization of facts.

The book accomplishes its purpose excellently.

E. V. N.

*Handbook of Diseases of the Skin* RICHARD L. SUTTON AND RICHARD L. SUTTON, JR. St. Louis, The C. V. Mosby Co., 1949 749 pp \$12.50

Here is an entirely new book by the Suttons, father and son, bringing to the number of four their texts on Dermatology.

Larger than their Synopsis, it is smaller than "Introduction to Dermatology," which in turn is much smaller than "Diseases of the Skin," the last edition of which appeared ten years ago.

The book contains 749 pages. It is well indexed and illustrated and contains a moderately complete up-to-date bibliography. The reader finds with ease what he is seeking.

The introductory chapters on the anatomy and physiology of the skin are well written, contain much that is new and interesting.

Departing from common dermatological procedure, an attempt has been made to present the subject matter from the etiological standpoint as far as possible,

and in some instances, I think, farther than is possible at the present time. Although in general the purpose and intent is excellent in this respect, the presented concept of that enormous group of eruptions commonly called, "dermatitis," or, "eczema," is unjustifiedly oversimplified, in a way that will assuredly lead astray the inexperienced and unwary student, who meets in other parts of the book such terms as, "eczematized dermatitis," "coccic dermatitis," etc. I still believe it is simpler to stick to the term, "eczema", when the cause is unknown, and, "dermatitis", when it (is) known, for that syndrome with which all experienced clinicians are familiar.

In this connection, the principles of allergy and sensitivity are more profoundly and fully dealt with than in any other text on Dermatology. Particularly stimulating is the inclusion of a paragraph on cutaneous autosensitization, even although the fundamental work of Burky with rabbit lens protein (Keratin), and rabbit muscle was not mentioned.

The internal medical viewpoint toward Dermatology is emphasized throughout in this book tying in Dermatology with General Medicine in a most commendable manner, not so evident in previous texts. This is true of the section on syphilis where the subject is covered so fully, that much of it is written in fine print, very trying on the eyes. This section is very definitely written for the specialist, requiring a rather thorough background knowledge of the disease for its proper evaluation and understanding.

Complete and concise coverage is given to the acute infectious diseases, with a wealth of new information on both the common and rare ones, domestic and tropical, caused by viruses, rickettsias, bacteria, and fungi.

The great value of modern treatment with anti-sera, sulfonamides, and antibiotics in the infectious diseases is brought out, unfortunately sometimes in an obscure fashion, due to the inclusion of older approaches in the discussions of treatment. The relative specificity of sulfapyridine in the treatment of dermatitis herpetiformis might have been stressed, since it is well known.

The treatment of Acne Vulgaris deserves special comment. The Suttons argue that the cause of Acne Vulgaris rests in part on the premise that overabundantly ingested fat finds its way outside the body by way of the hair follicles, thus causing them to become packed with sebum, which in turn leads to the picture we know as Acne, and that therefore this disease can be cured by lowering the fat intake and administering thyroid extract to lower lipemia.

H H H

*Hindu Medicine* By HENRY R. ZIMMER, Ph.D. Baltimore, The Johns Hopkins Press, 1948. 201 pp. \$4.00.

In November 1940, Henry Zimmer, distinguished student of Indian history and culture, delivered the seventh course of the Hideyo Noguchi Lectureship at the Johns Hopkins Institute of the History of Medicine. His subject was *Hindu Medicine*. Zimmer died before he finished revising his manuscript for publication, and this volume includes only the first two lectures. Zimmer described the primi-

tive medical lore of India, and the rationale of Hindu medicine. The education of the physician is depicted along with the difficulties encountered in the study of the basic source of anatomy. Each Hindu physician was instructed to ask himself four questions. First, are the complaints of the patient based on some real suffering or is he only seemingly ill? If he is ill, with what kind of suffering is the patient afflicted, and what is its origin? The third question is can the disease be cured or not? If it can, what kind of treatment is indicated for this particular ailment?

The lectures are introduced by a short biography of Zimmer by Ludwig Edelstein.

O D R

*Medical Writing* Second edition By MORRIS FISHBEIN Illus 292 pp \$4.00  
The Blakiston Company, Philadelphia, Pennsylvania, 1948

The usefulness of a guide to good medical writing need scarcely be argued. This book which represents the theory and practice associated with the conduct of the editorial department of the American Medical Association is one of the best on the subject. Its scope is indicated by some of the chapter headings: "An Acceptable Paper Style Construction of the Manuscript Words and Phrases Spelling Bibliographic Material Preparation of the Manuscript Illustrations Tables and Charts Revision Proofreading."

Any medical author who wishes to improve both the scientific value and the literary quality of his work will find this book most helpful.

E M H

*Parathyroid Glands and Metabolic Bone Disease* By FULLER ALBRIGHT AND EDWARD C REIFENSTEIN, JR 393 pp The Williams & Wilkins Company, Baltimore, 1948

The book is exactly what it purports to be, a compendium of the extensive experience and researches of the authors on diseases of bone, both with and without metabolic disease. There are many excellent x-ray reproductions, and the authors have used charts and diagrams liberally to portray the metabolic data. Theories and postulates, which are admittedly "as of today and subject to change" if and when data to the contrary may be elicited, are highly provocative. The book is written in a light, readable style and contains a wealth of information which should be highly useful to internist and orthopedist alike. The manuscript is excellently proof read, and documented with a wide bibliography.

J E H

*Posttraumatic Epilepsy* By A EARL WALKER 90 pages \$2.75 Illustrations Charles C Thomas, Springfield, Illinois, 1948

This small monograph is one of a series of American Lectures in Surgery given by authorities in different fields. The subject is one with which the author has had considerable clinical experience in this country, and to which he has contributed experimentally.

Whereas the monograph is based largely on his personal findings and experience, he has drawn wisely from the literature on the subject. Especially noteworthy is his rigid limitation of what constitutes "posttraumatic epilepsy." He has pointed out its relative rarity in the absence of gross evidence of previous severe, open, head injury. For many reasons, this long-known observation is constantly neglected by physicians and unrecognized by the laity. In such event, delayed recognition of non-traumatic lesions on the one hand, or unnecessary investigations on the other, may result. By the same token, he has wisely indicated the comparative infrequency with which surgical intervention should be undertaken in these cases. Good medical management is the basis of treatment. The results, in his and other hands, of augmenting such care in refractory cases by focal extirpation, are stated clearly.

Brevity has perhaps caused a seemingly restricted treatment of certain aspects of the subject. For the general physician, a more detailed description of the varied features of explosive attacks, and a consideration of diagnostic difficulties, would have added to the value of the book. Similarly, and exemplified strikingly by the author's own material, one might have expected a more complete consideration of the emotional features associated with this entity and with severe head injuries in general. These practical points, however, are minor criticisms of a clear and well-considered presentation of the current status of the "discharging focus."

J W M

*The Uses of Penicillin and Streptomycin* By CHESTER SCOTT KEEFER, M.D.  
University of Kansas Press, Lawrence, Kansas, 1949. 72 pp. \$2.00

This concise little book covers in a simple straightforward way the clinical uses of these two most common antibiotics. The book consists of three lectures which Dr. Keefer gave at the University of Kansas. The lectures have been apparently geared for the busy general practitioner who needs a ready guide to the indications for, methods of administration, and dosage of these two drugs. As such it is in most places very satisfactory. It does not go beyond the didactic and concise presentation which can be found in any of the drug house bulletins.

The third chapter is a quick historical sketch of the attempts at developing antibiotics which followed Pasteur's observation of the competition between bacteria, both in the test tube and in the animal body. This, too, is clear and in several places informative.

The book then is a satisfactory one, but not an essential one.

F B B

*Textbook for Almoners* By DOROTHY MANCHÉE, St Mary's Hospital, London  
The Williams & Wilkins Company, Baltimore. 452 pages. \$7.50

This book presents a brief history of hospital social work in England and a comprehensive picture of its community resources as of 1947. One gets a sense of the Almoner's continuing struggle to be relieved of routine duties so that she might be

able to offer more clearly defined services as a medico-social worker. Instead, a large portion of her time is spent with fee setting, follow-up, and numerous other time-consuming duties. It is not surprising that such a book, with its emphasis on the details of routine procedures, would have little remaining space for discussion of social work skills in a medical setting.

This, perhaps, would be a useful handbook for beginning Almoners, but would be of little value to Medical Social Workers in this country.

V M

## BOOKS RECEIVED FOR REVIEW

- A Primer of Electrocardiography* 2nd Edition By GEORGE E BURCH AND TRAVIS WINSOR 245 pp 265 illustrations, \$4 50 Published by Lea & Febiger, Phila 2, Pa
- Electrocardiography and Clinical Disorders of the Heart Beat* By SIR THOMAS LEWIS Published by Shaw & Sons Ltd Fetter Lane, London, England Pp 285, 25/-
- Handbook of Materia Medica, Toxicology and Pharmacology* By FORREST RAMON DAVISON 730 pp \$8 50 Published by C V Mosby Co, 3207 Washington Blvd, St Louis 3, Missouri
- Observations on the Pathology of Hydrocephalus* By DOROTHY S RUSSELL Published by His Majesty's Stationery Office, York House, Kingsway, London, W C 2 138 pp 6s net
- Studies and Research Concerning the Sheaf of the Natural Mannite* By DOMENICO GIGANTE (Direzione Generale de la Produzione Agricola Div 11) 144 pp
- The Uses of Penicillin and Streptomycin* By CHESTER SCOTT KEEFER, M.D. Published by The University of Kansas Press, Lawrence, Kansas 72 pp \$2 00
- Fundamentals of Internal Medicine* 3rd Edition By WALLACE MASON YATER 1451 pp \$12 00 Published by Appleton-Century-Crofts, Inc, 35 West 32nd Street, N Y 1
- Clinical Orthoptics* By ERNEST A W SHEPPARD, M D AND LOUISE WELLS-KRAMER 475 pp \$8 00 Published by C V Mosby Co
- Care of the Surgical Patient* by Jacob Fine 544 pp \$8 00 Published by W B Saunders, Phila, Pa
- Clinical Auscultation of the Heart* By SAMUEL A LEVINE AND W PROCTOR HARVEY 327 pp \$6 50 Published by W B Saunders, Phila, Pa
- Nutrition and Diet in Health and Disease* By JAMES S MCLESTER 5th Edition Published by W B Saunders, Phila, Pa 800 pp \$9 00
- Obesity* By RYNEARSON AND GASTINEAU 134 pp \$3 50 Published by Charles C Thomas, Springfield, Illinois
- Problems of Early Infancy* Transaction of 2nd Conference, March 1-2, 1948, New York Publication of Josiah Macy, Jr Foundation 120 pp \$1 00
- Conference of Metabolic Aspects of Convalescence* Transactions of 17th meeting, March 29-30, 1948, New York Publication of Josiah Macy, Jr Foundation 246 pp \$4 00
- Conference of Metabolic Aspects of Convalescence* Transactions of 16th meeting, Oct 27-28, 1947, New York Publication of Josiah Macy, Jr Foundation 168 pp \$3 00
- Clinical Cystoscopy* By LOWRAIN E MCCREA In two volumes, 2nd edition Published by F A Davis Co Philadelphia 3, Pa 1152 pp \$28 00





# DIAGNOSIS OF PORTAL VEIN OBSTRUCTION, STUDIES OF INTESTINAL ABSORPTION OF GLUCOSE USING ABDOMINAL COLLATERAL VEINS\*

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Patients with ascites frequently present problems in diagnosis. It is often difficult to determine whether the ascites is a manifestation of tuberculosis of the peritoneum, carcinomatosis of the peritoneum, partial or complete obstruction of the portal vein or other conditions accompanied by the presence of fluid in the peritoneal cavity. Evidence obtained from history, physical examination and laboratory investigations may not yield sufficient information upon which a definite diagnosis may be based.

The presence of collateral venous circulation in the abdominal wall of patients with ascites may at times be helpful in establishing the diagnosis of obstruction of the portal vein. But it is surprising how often malnourished individuals with abdominal distension and free fluid in the peritoneal cavity display a prominently visible network of veins of varying size over the wall of the abdomen. These prominent veins or venules may add to the confusion regarding a diagnosis.

In the presence of portal venous obstruction, the collateral circulation carries a part or all of the blood ordinarily consigned to the portal vein. The collateral veins may, therefore, be expected to transport the products of intestinal absorption circuitously around the obstruction.

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It was the purpose of this investigation to determine whether the products of intestinal absorption could be demonstrated in the collateral veins of the abdominal wall in patients with portal vein obstruction, and if so whether such a procedure might not assist in the differential diagnosis of the etiology of ascites

To this end the oral glucose tolerance test was employed. Blood was drawn at regular intervals after the ingestion of glucose, simultaneously from the antecubital vein and from a vein in the abdominal wall, and the blood sugar content measured

Sixteen patients with ascites were studied. One additional patient without ascites was studied in whom obstruction of the inferior vena cava and of the portal vein was suspected and later proven to be present at operation. Two patients with no ascites and in whom there was no reason to believe portal vein obstruction was present were also studied for the purpose of obtaining normal curves. Each patient was given 100 grams of glucose by mouth while in the fasting state. The abdominal veins were often so small that a tuberculin syringe with a 24 to 26 gauge needle was used. A small amount of blood was withdrawn and transferred to an indented paraffin block. One-tenth of a cubic centimeter of this blood was carefully drawn into a micro-pipette for sugar content determination using the micro-Folin-Wu technique. Blood was not drawn exactly at the same moment from the abdominal vein and the antecubital vein. It was drawn each time first from the abdominal vein and thereafter as soon as possible (one to three minutes) from the antecubital vein.

#### MATERIAL

The nineteen patients studied are listed in Table I with diagnoses and results of the simultaneous glucose tolerance tests. Patient #1 was a normal subject. Patient #2 had an obstruction of the superior vena cava with resultant development of large veins over the upper abdominal wall. There was no evidence of liver disease, portal vein obstruction, or ascitic fluid. Figure 1 demonstrates graphically the normal simultaneous glucose tolerance curves obtained in the study of patient #2.

*Patient #3* A 40 year old white male was admitted to the Thayer Veterans Administration Hospital in September 1946. He gave a history of anorexia, loss of

TABLE I

PA TIENT	DIAGNOSIS	GLUCOSE TOLERANCE TESTS														
		Milligrams sugar per cent in blood from														
		Arm vein									Abdominal wall vein					
		Hours									Hours					
		Fast	1/2	1	1 1/2	2	2 1/2	3	Fast	1/2	1	1 1/2	2	2 1/2	3	
1	Normal	71	117	123												
2	Obstruction superior vena cava (no portal obstruction)	101	147	161		80			70	61	120	118				
3	Cirrhosis of liver, central congestive type, biopsy	74	117	142		194	125			94	144	170		70	67	
4	Cirrhosis of liver, portal obstructive type				122				70	160	166		153			
5	Oct 3, '47—Biopsy, laparotomy, carcinoma of biliary tract, later (March 8, '48) of portal vein (autopsy)	80	96	131	135	132	132	131	80	131	200	197	195	202	202	
		94	101	118	117				96	217	185	147				
		105	174	163	136		72		102	163	154	118		97		
		85	94	102												
6	Obstructing cirrhosis of liver, portal obstructive type	120	172	191					89	128	141		131			
7	Chronic constrictive pericarditis with cirrhosis of liver, congestive type (autopsy)	108	150	144		190			148	108	187	200		211	171	
8	Cirrhosis of liver, portal obstructive type	91	171	169	143	143			99	169	185		170			
9	Cirrhosis of liver, portal obstructive type	97	144	177		160			89	150	182	169	163			
10	Cirrhosis of liver, portal obstructive type	94	141	143		140			167	97	139	187		178	177	
11	Carcinomatosis of peritoneum, primary carcinoma of ovary (exploratory laparotomy)	90	139		174	184			106	100	160	156		140	106	
12	Intestinal lipodystrophy (Whipple's disease) exploratory laparotomy	89	119		108	101			91	133		179		179		
13	1) Cirrhosis of liver, portal obstructive type 2) Tuberculous peritonitis (autopsy)	77	122	133		103	104		85		125		111	96		
									91	117	147		108	108		

185

TABLE 1—*Continued*

PA TIENT	DIAGNOSIS	GLUCOSE TOLERANCE TESTS													
		Milligrams sugar per cent in blood from													
		Arm vein							Abdominal wall vein						
		Hours							Hours						
		Fast	½	1	1½	2	2½	3	Fast	½	1	1½	2	2½	3
14	Tuberculous peritonitis (exploratory laparotomy)	91	133		161		126		84	118		158		138	
15	Carcinoma of ovary with peritoneal metastases (exploratory laparotomy)	91	170	206	217	222	206		93	172	187	200	198	200	
16	Rheumatic heart disease, chronic general failure of circulation	98	127		156	125		101	90	114		149	132		123
17	Chronic congestive heart failure	85	120	128		132		112	96	119	148		133		115
18	Cirrhosis of liver, portal obstructive type	93	174	182	167	145			102	143	182	174	163		
19	Carcinoma obstructing inferior vena cava and extrahepatic portal vein	88	117	149	130	114	92	70	82	138	166	150	126	102	79

weight, mild watery diarrhea and indigestion accompanied by gradually increasing abdominal distension over a two months' period. He denied excessive consumption of alcohol. Physical examination revealed wasting, slight jaundice, ascites, palpable liver, and prominent superficial abdominal veins. There was no evidence of cardiovascular disease. Laboratory investigations revealed diminution of liver function with decreased total serum protein and an icterus index of 18. An exploratory laparotomy was performed and a specimen of the liver obtained. On microscopic examination there was irregular fibrosis of the parenchyma associated with intense engorgement of the veins. A diagnosis of central or congestive type of cirrhosis was made. It was believed that obstruction of the hepatic vein, cause undetermined, was present, with resultant increased pressure in the portal vein. In July 1947 the glucose tolerance tests were performed. Sugar values were consistently higher in the specimens of blood obtained from the abdominal veins than in those from the antecubital vein, Figure 2. These data were interpreted as indicating the presence of definitely increased pressure in the portal vein.

*Patient # 4* A 48 year old white male was admitted to the Thayer Veterans Administration Hospital in July 1947 with a history of anorexia for six weeks, vomiting, and the onset of abdominal distension one week prior to admission. He had consumed large amounts of alcohol for two years prior to admission and had

had one episode of delirium tremens. On physical examination there was abdominal distension, ascites, enlarged liver and slight edema of the ankles. He was jaundiced. Laboratory investigations revealed evidence of liver damage. Total serum protein was 6.4 grams per cent with 3.0 grams of albumin. Figure 3 demonstrates graphically simultaneous glucose tolerance curves the first, July 21, 1947, the

## PATIENT # 2 NORMAL

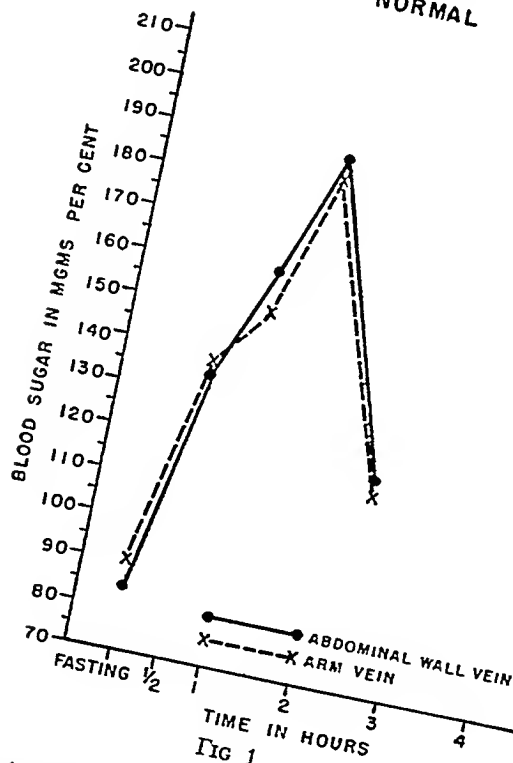


FIG 1

second, October 6, 1947. These curves present strong evidence in support of a diagnosis of portal cirrhosis of the liver.

**Patient # 5** A 25 year old white truck driver was admitted to Thayer Veterans Administration Hospital July 17, 1947 with a history of indigestion and pain below the right costal margin of six weeks duration, jaundice for five weeks, and vomiting for two weeks. The patient was markedly jaundiced. The liver was large, smooth and tender. Laboratory investigations revealed evidence of obstructive jaundice. Shortly after admission ascites was noted. On September 29, 1947 a biopsy of the

liver provided evidence of biliary cirrhosis. In October 1947 an exploratory laparotomy was performed and carcinoma of the biliary tract was demonstrated. In November 1947 an unsuccessful attempt was made to relieve the biliary obstruction. At this time the entire hilus of the liver was found to be involved in a carcinomatous mass. Autopsy examination in the latter part of March 1948 revealed complete occlusion of the portal vein by widespread adenocarcinomatosis. Figure 4

**PATIENT #3**  
**CIRRHOSIS OF LIVER**  
**CONGESTIVE TYPE**

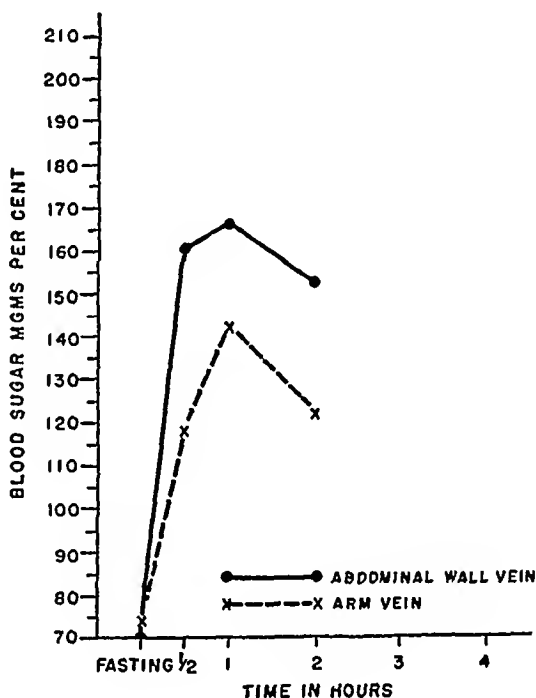


FIG 2

demonstrates graphically two pairs of simultaneous glucose tolerance curves, one made before and the other after the development of portal obstruction. At the time of the laparotomy on October 3, 1947, there was no demonstrable evidence of portal obstruction. During the five months' period between the two tests, there had been no change in the clinical picture to indicate that portal obstruction had developed. This information, therefore, was obtained prior to the patient's death only by the use of the simultaneous glucose tolerance tests.

*Patient # 6* A 53 year old white coal miner was admitted to Thayer Veterans Administration Hospital December 7, 1947 with a history of intermittent pain in the right upper quadrant of the abdomen for six years and abdominal distension for two months. He had consumed large amounts of alcohol for many years and had always eaten poorly. His abdomen was distended, there were a few distended superficial abdominal veins and the area of liver dullness was increased. There was ascites. Laboratory investigations revealed evidence of liver damage. Total serum

PATIENT # 4  
PORTAL CIRRHOSIS OF LIVER

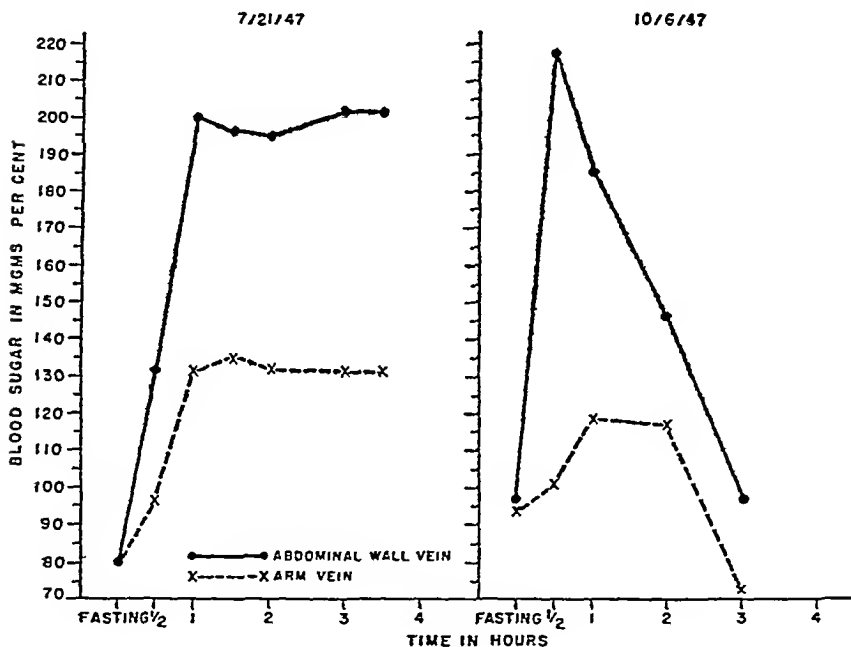


FIG 3

protein was 5.6 grams with 2.9 grams of albumin. Simultaneous glucose tolerance tests gave blood sugar values shown in Table I which are interpreted as indicative of portal vein obstruction.

*Patient # 7* A 28 year old colored laborer was admitted to Nashville City Hospital with a history of progressive abdominal distension, shortness of breath and swelling of the ankles. At autopsy he was found to have constrictive pericarditis which chiefly involved the entrance of the inferior vena cava into the right auricle. There were large superficial abdominal veins. The liver was large and there was marked congestive fibrosis. The simultaneous glucose tolerance tests revealed



evidence of increased pressure in the portal veins, Figure 5 This observation in a patient with constrictive pericarditis with, in effect, partial obstruction of the inferior cava above the liver, is of special interest because of the fact that the simultaneous glucose tolerance curves of two patients, # 16 and 17, with ascites associated with chronic congestive heart failure did not give evidence of increased pressure in the portal vein above that of the systemic veins Moreover, this is in

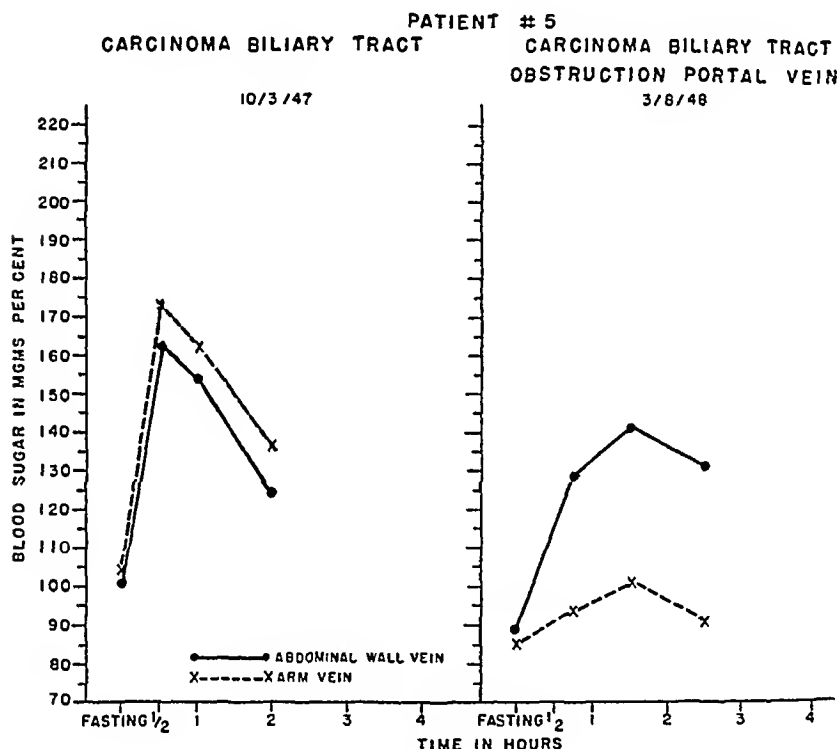


FIG 4

contrast to the normal values obtained in the study of Patient # 2 whose superior vena cava alone was obstructed

*Patient # 8* This 52 year old white carpenter gave a history of recent indigestion and abdominal distension in association with chronic long-standing alcoholism Physical examination and laboratory investigations indicated the presence of cirrhosis of the liver The simultaneous glucose tolerance tests gave corroborative evidence of some portal vein obstruction, Table I

*Patient # 9* A 57 year old grocery clerk was admitted to Thayer Veterans Administration Hospital March 2, 1948 complaining of indigestion and abdominal

distention. He had consumed large amounts of alcohol and had a poor appetite for many years. There was ascites, some distention of superficial abdominal veins and a palpable liver which seemed to be nodular. Laboratory investigations revealed evidence of liver damage. Total serum protein was 6.1 grams per cent with 2.9 grams of albumin. The simultaneous glucose tolerance curves in Figure 6 gave suggestive but not conclusive evidence of some portal vein obstruction.

**PATIENT # 7**  
**CONSTRUCTIVE PERICARDITIS**

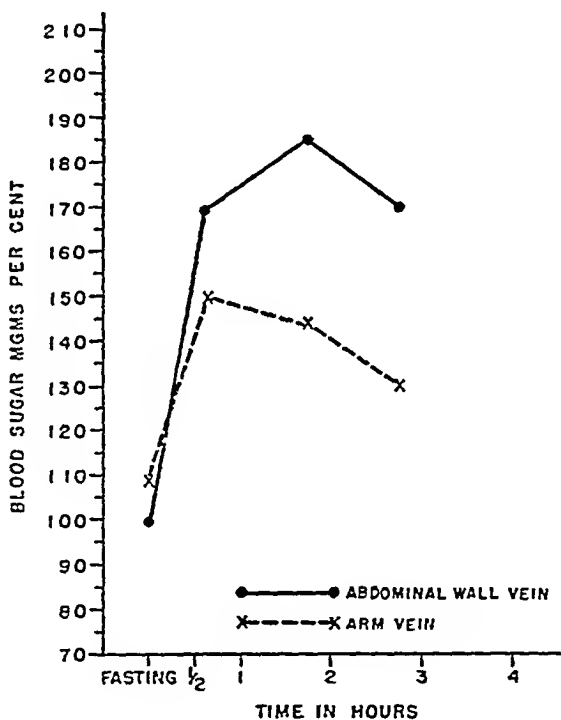


FIG 5

*Patient # 10* A 36 year old white housewife was admitted to Vanderbilt University Hospital February 2, 1948 complaining of intermittent abdominal distention and indigestion for seven months. She denied excessive consumption of alcohol. She had been treated for syphilis thirteen years ago over an eighteen month period. Physical examination revealed evidence of weight loss, several spider angiomas, abdominal distention, ascites, and although the liver was not palpable the area of liver dullness was enlarged to percussion. The Wassermann

test was positive. Laboratory investigations revealed evidence of diminished liver function. Total serum protein was 5.9 grams per cent with 2.02 grams of albumin. Simultaneous glucose tolerance test gave blood sugar values, shown in Table I, which were not conclusive. Although this patient almost certainly had Laennec's cirrhosis of the liver, portal hypertension may not have been present. If collateral

**PATIENT # 9**  
**PORTAL CIRRHOSIS OF LIVER**

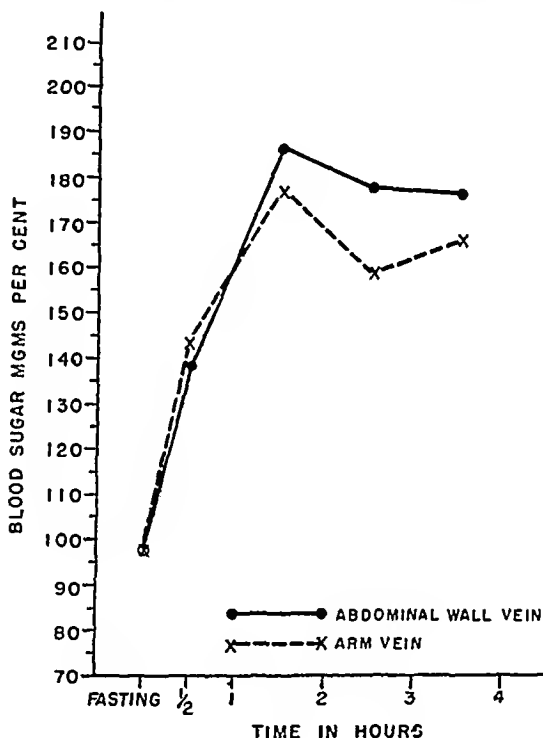


FIG 6

circulation was present it was not demonstrable in the veins of the anterior abdominal wall.

*Patient # 11* A 43 year old housewife was admitted to Vanderbilt University Hospital complaining of progressive abdominal distension over a four months' period. Physical examination revealed ascites and deviation of the cervix to the left. There was a hard nodule palpable in the cul de sac on rectal examination. Exploratory laparotomy was performed and widespread adenocarcinomatosis originating in the right ovary with metastases throughout the peritoneal cavity

was demonstrated. Simultaneous glucose tolerance tests carried out prior to operation yielded data which indicated that there was no portal vein obstruction, Table 1. This was confirmed at operation.

*Patient # 12* A 47 year old farmer was admitted to Vanderbilt University Hospital on October 18, 1947 complaining of upper abdominal pain, shortness of breath, diarrhea and abdominal swelling. He had had indigestion and abdominal

# PATIENT # 12 INTESTINAL LIPODYSTROPHY

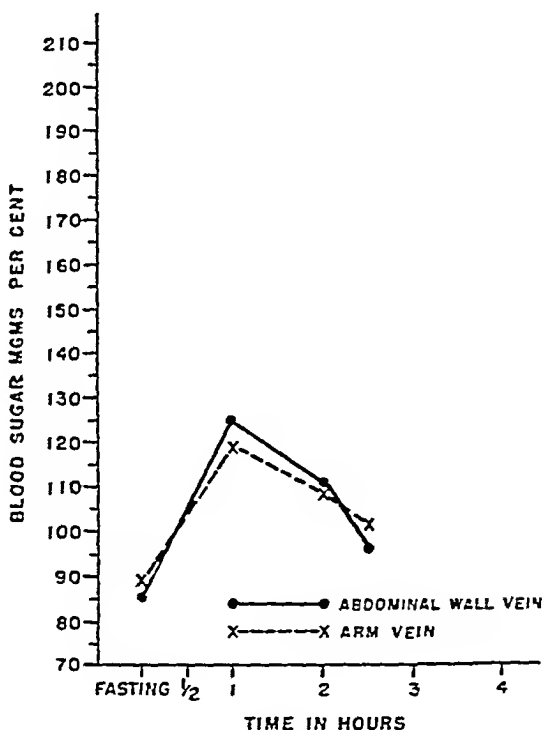


FIG 7

cramps for four and a half years and for two months had noted gradual increase in the size of his abdomen. He had abstained from alcohol for eight years, although prior to that time he had been a moderately heavy drinker. He had lost forty pounds during the present illness. On physical examination there was evidence of weight loss, cachexia, scattered petechiae over the skin of the legs, abdominal distension with ascites, enlarged liver and spleen. There was no evidence of collateral circulation. Laboratory investigations revealed very little evidence of liver disease.

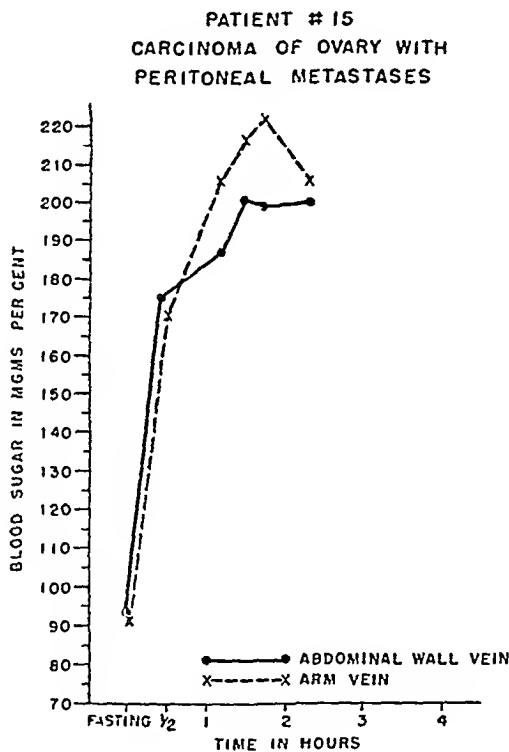
Total serum protein was 4.63 grams per cent with 2.6 grams of albumin. In spite of the lack of confirmatory laboratory data, it was the opinion of those who examined him that this patient had cirrhosis of the liver with portal vein obstruction. Simultaneous glucose tolerance tests failed to produce evidence of portal vein obstruction, Figure 7. Following paracentesis a mass thought to be retroperitoneal was felt to the left of the midline in the upper abdomen. An exploratory laparotomy was performed on November 24, 1947. The typical picture of intestinal lipodystrophy was observed and was confirmed on microscopic examination of a mesenteric lymph node. There was no evidence of portal vein obstruction.

*Patient # 13* This patient was a 60 year old housewife who was closely followed at Vanderbilt University Hospital from May through October 1947. She was admitted three times for investigation and finally an exploratory laparotomy was performed October 2, 1947. She died ten days later because of a cerebrovascular accident. Her course was characterized by the development of indigestion, anorexia, and progressive abdominal distension and weight loss over a period of six months prior to admission to the hospital. She was cachectic, and there was abdominal distension and ascites. Laboratory investigations revealed evidence of liver disease. Paracenteses consistently yielded a turbid pseudochylous fluid. Serologic tests for syphilis were positive. At operation, numerous enlarged tuberculous mesenteric lymph nodes were observed. The lymphatic vessels draining the intestines were obstructed and engorged. Moreover, there was nodular portal cirrhosis of the liver. Prior to operation simultaneous glucose tolerance tests, yielded blood sugar values which are shown in Table I. The results were not conclusive. At operation two factors, tuberculous peritonitis and cirrhosis, one or the other or both of which could have been responsible for the ascites, were found.

*Patient # 14* This 41 year old housewife was admitted to Vanderbilt University Hospital in September 1947 complaining of progressive abdominal distension, weakness and loss of weight. She denied the use of alcohol. The family history was noteworthy in that several close relatives had died of tuberculosis. Physical examination revealed evidence of weight loss, abdominal distension, the presence of ascites and hepatomegaly. The superficial abdominal veins were prominent. Although laboratory investigations revealed no evidence of liver dysfunction, the presence of an enlarged liver, ascites and prominent abdominal veins seemed to point to a diagnosis of Laennec's cirrhosis of the liver. Some observers were of the opinion that the patient had tuberculous peritonitis. Acid fast bacilli were not demonstrated in the ascitic fluid, the specific gravity of which was border line between that of a transudate and an exudate. Simultaneous glucose tolerance tests, Table I, showed no evidence of portal vein obstruction. Exploratory laparotomy revealed extensive tuberculous peritonitis. There was no obstruction of the portal vein.

*Patient # 15* This 40 year old housewife was admitted to Vanderbilt University Hospital first in May 1947 complaining of abdominal distension, loss of weight and weakness for the preceding three years. The course of her illness was marked by its chronicity and by the remarkable disappearance and reappearance of ascites.

Although tests of liver function were for the most part within normal limits, a diagnosis of Laennec's cirrhosis was made. She had always had poor dietary habits. She denied the use of alcohol, but had consumed large amounts of turpentine orally for contraceptive purposes. Simultaneous glucose tolerance curves, Figure 8, revealed no evidence of portal vein obstruction. Finally in October 1948, following a paracentesis, asymmetry of the abdomen was noted and on pelvic examination a mass in the region of the left ovary was felt. Extensive peritoneal metastases



from an ovarian cystadenocarcinoma were demonstrated when an exploratory laparotomy was performed in November 1948. The portal and mesenteric veins were carefully examined. They were normal.

*Patient # 16* This 28 year old white male was continuously confined to bed in Vanderbilt University Hospital for almost a year. He had had acute rheumatic fever as a child with resultant mitral insufficiency and stenosis. There was marked chronic congestive heart failure, and persistent ascites, associated with massive peripheral edema. Simultaneous glucose tolerance tests revealed no evidence of

portal vein obstruction as shown in Table I Examination at autopsy revealed a huge congested liver with the typical changes associated with chronic passive congestion due to heart failure

*Patient # 17* This 60 year old colored female had been followed for many years at Vanderbilt University Hospital She developed congestive heart failure on an

# PATIENT # 18 PORTAL CIRRHOSIS OF LIVER

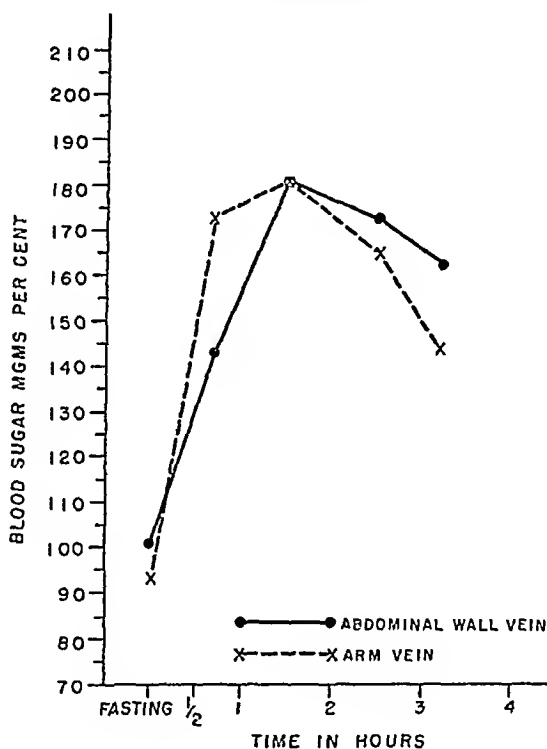


FIG 9

arteriosclerotic basis with enlargement of the liver and ascites Simultaneous glucose tolerance tests, Table I, revealed no evidence of portal vein obstruction

*Patient # 18* A 59 year old white male was first admitted to Vanderbilt University Hospital with tremendous abdominal distension which had gradually become more and more apparent for six months prior to admission He had periodically consumed large amounts of beer and whiskey and had had a very poor diet for many years He gradually became weaker, eventually lost his appetite altogether and on admission was completely exhausted He was a rather thin man ex-

cept for his huge abdomen, the maximum circumference of which measured six feet. The abdominal wall was very tightly stretched by the large amount of fluid present in the peritoneal cavity. Laboratory investigations revealed marked impairment of liver function. Total serum protein was 6.9 grams per cent with 3.42 grams of albumin. Although there was convincing evidence that this patient had Laennec's cirrhosis of the liver with portal vein obstruction, the simultaneous glucose tolerance curves were not significantly different one from the other, Figure 9. It is probable that the marked intra-abdominal pressure and distension of the abdominal wall were such as to result in raising the pressure in the veins of the abdominal wall above that of the portal vein. Under such circumstances collateral circulation could not be maintained through the abdominal veins. Unfortunately the validity of this explanation could not be tested by repeating the examination after the removal of a quantity of ascitic fluid, thus lowering the intra-abdominal pressure and abdominal wall tension. No veins were visible in the abdominal wall after paracentesis.

*Patient # 19* A 47 year old white farmer was admitted to Vanderbilt University Hospital on January 24, 1949 complaining of pain in the flanks, weakness and weight loss. He was thin, there was marked dilatation of the veins of the abdominal wall, the liver was enlarged and there was a firm fixed mass palpable deep in the abdomen above the umbilicus. There was brawny induration of the skin and subcutaneous tissues of the feet and ankles. Venous pressure in the arm was sixty millimeters of water. In the lesser saphenous vein of the leg the pressure was one hundred thirty five millimeters of water. A tentative diagnosis of retroperitoneal malignancy with obstruction of the inferior vena cava was made. Simultaneous glucose tolerance tests were carried out. Sugar levels in the blood obtained from the vein of the abdominal wall were consistently higher than those in blood from the antecubital vein, Table I. On this basis, extrahepatic obstruction of the portal vein was suspected in addition to obstruction of the inferior vena cava. At operation, the mass was discovered to be composed of matted lymph nodes and omentum through which the portal vein and its tributaries passed. The pressure in the portal vein above the lesion was eighty millimeters of water, below the mass in the superior mesenteric vein, the pressure was two hundred eighty millimeters of water. This seemed to be confirmatory data for the clinical diagnosis of portal vein obstruction. The inferior vena cava was not visualized. Microscopic examination of the biopsy material revealed metastatic carcinoma of undetermined origin. No site of origin was discovered before the patient was discharged from the hospital.

#### DISCUSSION

Sherlock and Walshe (1) reported the results of studies of intestinal absorption of carbohydrate, fat and amino acids in a patient with cirrhosis of the liver in whom an enlarged abdominal wall vein communicated with the portal venous system. The patient did not have ascites but the data obtained from clinical history, physical examination,



laboratory investigations and a biopsy of the liver conclusively supported a diagnosis of Laennec's cirrhosis. These investigators were able to demonstrate higher blood sugar values in the abdominal wall than in the antecubital vein after administration of oral glucose. They concluded from this data that the vein in the abdominal wall carried blood from the mesenteric veins.

We were able to duplicate some of the results of Sherlock and Walshe. Moreover the procedure appears to offer a practical adjunct to other methods of diagnosing portal vein obstruction regardless of the etiology.

The procedure may occasionally be rather tedious and time consuming. Abdominal veins, even in the presence of portal vein obstruction, are often small and multiple samples of blood may be obtained from them only with difficulty.

Several factors may tend to make results misleading or inconclusive. Collateral circulation in the presence of portal vein obstruction may not develop through the veins of the abdominal wall. When distension or ascites are present, the pressure in the superficial abdominal veins may actually be higher than in the portal vein itself. Moreover, admixture of systemic blood in the abdominal veins may be sufficient to obscure the presence of a small increase in blood sugar levels. Nevertheless, our experience indicates that the procedure is often helpful and sometimes diagnostic.

Interpretation of the blood sugar curves does not always lead to clear conclusions. There are variations between fasting sugar levels in the blood of the same patient taken almost simultaneously from the antecubital and abdominal wall vein which can only be accounted for by the difficulties frequently encountered in obtaining blood from small abdominal veins. But if the sugar levels in the blood obtained from the abdominal wall veins are consistently higher than those in the blood from the antecubital vein throughout the test, the results may be significant. For example, if the average elevation of sugar level in blood from the abdominal wall vein is at least fourteen milligrams per cent higher than in blood from the antecubital vein for four determinations made during the one to three hours after ingestion of glucose, the difference is statistically significant, and may be taken to indicate shunting of portal blood through the veins of the abdominal wall.

(The average elevation based on two successive readings would have to be at least twenty milligrams per cent higher and the average elevation based on three successive readings would have to be at least sixteen milligrams per cent higher to be statistically significant ) This was apparently true in seven of the eleven cases here reported in which a diagnosis of portal vein hypertension was compatible with clinical and anatomical findings (patients #3, 4, 5, 6, 7, 8, 19) In patients #9, 10, 13 and 18 in whom portal cirrhosis was present, results were inconclusive Of the eight patients in whom portal hypertension was not compatible with clinical and anatomical findings (patients #1, 2, 11, 12, 14, 15, 16 and 17 and in addition the first studies of patient #5), there was no statistically significant elevation of sugar levels in blood from the abdominal wall veins over levels in blood from the antecubital vein

#### SUMMARY

Simultaneous measurements of blood sugar levels in the antecubital and superficial veins of the abdominal wall of sixteen patients with ascites and one with portal vein and inferior vena caval obstruction without ascites, were made at intervals following the ingestion of glucose Two additional patients were used as controls In the presence of hypertension of the portal vein, higher levels of sugar were often demonstrated in blood from the veins of the abdominal wall than in blood from the antecubital vein Our experience indicates that this procedure is frequently helpful in determining whether or not portal vein obstruction is present

The authors wish to acknowledge the assistance rendered by Doctor Margaret P Martin in statistically interpreting the blood sugar curves

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# TREATMENT OF CUTANEOUS CARCINOMA WITH PODOPHYLLIN\*

## PRELIMINARY NOTE

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The purpose of this communication is to report brief observations of the manner in which cutaneous carcinomas undergo involution following the topical action of podophyllin

Podophyllin produces arrest of mitosis, distortion of nuclear pattern and a chain of cytotoxic effects (1) (2) (3) in the epithelium of normal skin as well as in the epithelial cells of any benign or malignant skin tumors with which it comes into contact for 8 hours or longer (1) (4) (5) There is abundant clinical (6) (1) and microscopic evidence (1) (2) (9) that the drug's cytotoxic effects are more selective for certain types of epithelial cells, particularly those of moist, young, non-keratinized condylomata acuminata which are rapidly destroyed by a single application By contrast the action on surrounding normal skin, unless excessive and unduly prolonged, is reversible (1) (2) (4) as macroscopic and microscopic integrity of the skin is established soon after removal of the resin Selective damaging effects on mouse tumors (7) and on mouse tumor cells in tissue culture (8) result from exposure to the drug Podophyllin and podophyllotoxin are more toxic for recently weaned mice than for adult mice (5), the toxin is extremely toxic for chick embryos (5) Dry keratinized verrucae vulgares and chronic, or old, partially keratinized condylomata acuminata are resistant to the rapid tumor necrotizing action The aforementioned facts indicate that the cellular destructive action is more selective for young, embryonic and tumor cells than for adult cells and that the changes produced in adult cells are reversible within certain limits

\* Work done wholly under a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council

The cancericidal potentialities were first pointed out when the similarity of colchicine and podophyllin effects was discovered (9). As a prelude to the clinical use of podophyllin for cutaneous carcinoma, studies in this laboratory (5) have accumulated sufficient data during the past 2 years to show that the topical application of podophyllin for prolonged periods is safe, provided contact with the eyes is avoided. The response of cutaneous carcinoma is slower than that exhibited by condyloma acuminatum and much more of the drug is required, but it appears from preliminary observations to be equally as impressive and as complete. Thus far 15 patients having a total of 25 cutaneous carcinomas have been treated. Biopsies were secured from each patient prior to treatment. Follow-up of 5 lesions was not accomplished because serial sections were excised at various stages of involution for microscopic analysis of treatment effects. Of the 20 lesions which were destroyed by multiple applications and permitted to heal, all except one may be considered satisfactory results. In this group there were 12 basal cell carcinomas measuring 0.5 cm, 0.8 cm, 1.5 cm, 1.5 cm, 1 x 2 cm, 2 cm, 2 cm, 2 x 2.5 cm, 2 x 3 cm, 2 x 3 cm, 2.5 x 3 cm and 12 x 14 cm, 7 intra-epidermal carcinomas measuring 2 cm, 2 cm, 3 cm, 4 cm, 4 cm, 4 x 6 cm and 8 x 14 cm, and 1 prickle cell carcinoma, 4 x 6 cm. The 12 x 14 cm basal cell carcinoma was the only failure in the group. The resin was applied topically as a 25 percent suspension in mineral oil or a 20 percent solution in alcohol. Numerous treatment schedules were tried, these varied from 2 applications in 7 days to 50 applications in 3 months. To date a satisfactory average schedule has not been determined but probably approximately 10 to 20 applications within 3 weeks will be adopted as a working schedule based on current trial and error results. The periods of involution of treated tumors varied considerably. The most rapid disappearance was in 10 days, the slowest was 3 months. It must be emphasized however, that varying experimental treatment schedules were being observed and that over treatment in more than half of the cases undoubtedly prolonged healing in some cases. Including such variables the average healing time was 49 days after the first application. A most impressive clinical observation is the selective ulceration of tumor tissue, the borders of treated basal cell carcinomas are channeled by the drug and the delineation of ulcerative moats

outlining previous tumor sites is clear cut in contrast to the superficial and reversible signs of injury to the surrounding normal skin. The treated area heals with a faint, slightly atrophic scar which resembles the cosmetically successful roentgen irradiation scar when it occurs without accompanying telangiectasia. The follow-up periods vary from one to eight months with an average of approximately six months.

The results herein reported are encouraging, but it is obviously premature to express enthusiasm for the method until sufficient time has elapsed to determine whether the results will be permanent. Toxicologic, pharmacologic and pathologic studies are being conducted (5) in conjunction with clinical studies to ascertain the tolerance of the drug, the time factors and amount of drug necessary to destroy cutaneous cancer permanently without causing irreversible damage to circumjacent normal tissue and at the same time avoiding hypersensitivity which is frequently produced by the drug (1) (10). The selective topical effects of podophyllin differ from the effects of other chemicals such as those utilized in the chemosurgical method of Mohs, which produces non-selective tissue destruction that is measured by microscopic examinations of the fixed and damaged tissues as they are removed. The anticipated objections to the use of podophyllin are two features that may be viewed by some as advantages, namely, simplicity and economy. The lack of available fixed tissue for microscopic examination during treatment may be an objection voiced by advocates of Mohs' chemosurgical microscopically controlled method. Therefore, it is not the intention of the author to recommend podophyllin as a treatment of carcinoma to supercede other well known methods such as surgery, roentgen therapy, electrodesiccation, cauterization or chemosurgery. It is hoped that other investigators may explore this method, duplicating our approach (4) to the problem, which consists of excising treated tumors in various stages of involution and after healing to determine sequential microscopic signs of the tumor necrotizing effect and the permanency of the destruction. Detailed descriptions of our findings (4) will be published next year after we have accumulated sufficient clinical and microscopic data to justify proper evaluation of the method.

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Addendum—Two other preliminary reports of the use of podophyllin in the treatment of cutaneous carcinoma and senile keratoses have been submitted recently by Dr Leslie Smith of El Paso, Texas and Dr Fletcher Hall of Los Angeles, California to the Archives of Dermatology and Syphilology The results of Smith and Hall (In Press) are essentially similar to those herein reported

M S

# A COMPARISON OF SARCOIDOSIS AND TUBERCULOSIS WITH RESPECT TO COMPLEMENT FIXATION WITH ANTIGENS DERIVED FROM THE TUBERCLE BACILLUS

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The etiology of sarcoidosis has not been established although an imposing number of observers have proposed arguments in favor of the theory that it is a form of tuberculosis. Several reviews in the past few years have discussed the principal evidence for and against this theory (1-8). The most significant facts to date regarding the possible relationship of these diseases are the following:

There is a striking similarity in the pathological lesions of sarcoidosis and tuberculosis. The epithelioid-cell tubercle is the characteristic histological unit of each. The lack of caseation in the lesions of sarcoidosis is the pathological feature that distinguishes it most clearly from typical tuberculosis. However, caseation does not invariably occur in the lesions of tuberculosis (9) so that its absence from the lesions of sarcoidosis is not an absolute differentiation. Moreover, it has been reported that patients with sarcoidosis react to the inoculation of heat-killed (10) or avirulent (11) tubercle bacilli with the formation of noncaseous tubercles in contrast to normal individuals in whom caseation occurs. Since caseation in tuberculous lesions is dependent in large part upon hypersensitivity of the tissues to tuberculo-protein, its failure to occur in sarcoidosis might conceivably be related to the fact that the great majority of cases of sarcoidosis are not hypersensitive to tuberculin. It has been reported, furthermore, that patients with sarcoidosis fail to develop hypersensitivity to tuberculin following vaccination with avirulent tubercle bacilli (11, 12, 13) as normal individuals do. There are a number of other clinical and pathological features of sarcoidosis that are not typical of tuberculo-

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sis (1-9) but the most persuasive evidence against a tuberculous etiology is the repeated failure of the great majority of investigators to identify the tubercle bacillus in the lesions of the disease by staining, culture and inoculation of tissue into a variety of animal species (1, 9, 14) Nevertheless an impressive proportion of fatal cases of sarcoidosis have proven to have typical tuberculosis at autopsy as well as typical sarcoidal lesions (1, 2, 5, 6, 8) The coexistence of both diseases in the same patient has been invoked by some as evidence of their identity and by others as evidence of their distinct individuality Some more specific evidence is needed before the etiological relationship of these diseases can be established

Antibodies to *M. tuberculosis* have been identified in the serum of tuberculous patients with considerable frequency (15, 16, 17, 18) Antibodies may also be demonstrated in the serum of infected animals throughout the course of infection and often persist when demonstrable skin sensitivity to tuberculin has waned (19) If sarcoidosis were in fact due to the tubercle bacillus it might be anticipated that antibodies to the organism would be found in these patients even in the absence of tuberculin sensitivity The present study was undertaken in order to determine whether complement-fixing antibodies to *M. tuberculosis* occur in the serum of patients with sarcoidosis with sufficient frequency to indicate an etiological relationship of this agent to the disease

#### CASE MATERIAL

A series of twenty-two cases of sarcoidosis has been tested The diagnosis was supported in each instance by biopsy of at least one involved tissue There were no autopsied cases In only one case, to be described below, was any bacteriological evidence of tuberculosis found during the course of this study The distribution of the lesions, demonstrated by clinical, roentgenographic and pathological means, was similar to that found by others in larger series of cases (6, 20, 21) The positive observations are recorded in Table 1 Every patient had a roentgenogram of the chest Enlarged hilar lymph nodes and pulmonary infiltrations were considered evidence of the presence of the lesions in these sites The pulmonary lesions were usually of miliary type but occasionally they were patchy and asymmetrical No cavities



TABLE 1  
Pertinent clinical, radiological and pathological data on the cases of sarcoidosis

CASE NUMBER		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Age (yrs)		29	23	48	25	25	34	26	47	19	24	24	19	32	30	31	49	28	22	65	35	21	44
Sex		F	F	F	M	M	M	M	F	M	M	M	M	F	F	M	F	M	M	F	F	F	F
Race		W	W	W	N	N	W	N	W	N	N	N	N	W	N	W	N	N	N	W	N	N	W
Tuberculin test (mg Old Tuberculin)	Positive									±			0 1							0 1	1 0	1 0	
	Negative	1 0	0 1	0 1	0 1	0 1	10	10	10	1 0	10	10		10	1 0	1 0			0 1				1 0
Guinea pig inoculation*		Neg				Neg	Neg	Neg	Neg	Neg		Neg	Neg	Neg	Neg	Neg	Neg	Neg		Neg	Neg	Neg	
Distribution of lesions	Lymph nodes																						
	Superficial§					+	+	+	+		+	+	+		+	+	+	+	+	+	+	+	+
	Mediastinal†			+	+						+	+	+		+	+		+	+	+	+	+	+
	Abdominal§																						
	Lungs†																						
	Lyes†																						
	Skin§			+	+	+	+	+	+														
	Parotid gland§			+	+	+																	
	Bones†																						
	Mucosae§																						
	Spleen§		+																				+

\* Inoculation with portion of biopsy material which contained lesions characteristic of sarcoidosis

† Diagnosed by roentgenographic examination

‡ Diagnosed by ophthalmoscopic examination

§ Lesions verified by pathological examination of biopsies

or apical lesions typical of tuberculosis were found. The lesions of the eyes consisted of active or healed uveitis and one case had in addition a retrobulbar neuritis. The biopsies demonstrated numerous epithelioid cell tubercles with no more than minimal microscopic necrosis. Carbol-fuchsin stains in every instance failed to reveal acid-fast bacilli. Portions of the biopsy material from 16 cases were inoculated into guinea pigs and none of the animals developed tuberculosis. With one exception no acid-fast bacilli were obtained from any source in this group of patients. The exception was a 19 year old negro youth (case 9) with typical uveoparotid fever, enlarged mediastinal lymph nodes and clear lung fields. His tuberculin test to 1 mg. of Old Tuberculin was equivocal. Smears of his sputum had been negative but one guinea pig, inoculated with material from a gastric lavage, developed tuberculosis. A portion of his parotid gland, which contained lesions typical of sarcoidosis, was inoculated into two other guinea pigs and yielded a negative result. The patient moved away before the gastric lavage could be repeated.

Records of tuberculin tests were available in all but two of the cases. The results are summarized in Table 1. None reacted to 0.01 mg. dose of Old Tuberculin. Two of the 16 who were tested with 0.1 mg. gave positive reactions. Two others who were tested with 1.0 mg. reacted. Five who were tested with 10 mg. were negative. Guinea pigs were inoculated with biopsy material from every case which had a positive tuberculin test.

The ages of the patients ranged from 19 to 65 years and the mean age was 28 years. There were equal numbers of men and women. Thirteen cases (59%) were negroes.

For comparison with the cases of sarcoidosis, a group of 26 cases of active tuberculosis was selected. This group included cases with pulmonary, renal, osseous and meningeal infection. The diagnosis in each instance had been confirmed by the demonstration of acid-fast bacilli in the sputum or other material. Only one of these cases had a negative tuberculin test. This case will be referred to again later. Further comparison was made with two groups of healthy preclinical medical students who had recently been tuberculin tested. The first group of 30 students reacted positively to the first test dose of PPD. Each one had recently had an x-ray of the chest and none had clinical or

roentgenographic evidence of active tuberculosis. The second group of 29 students was negative to the second test dose of P P D and none had a history of tuberculosis or of a previously positive tuberculin test.

#### METHODS

There is a large body of data on the complement fixation reaction in patients with tuberculosis. The wide variation in the results of early investigators (18) has been greatly reduced in the more recent studies employing better standardized methods (22). In 1927 Pinner (15) summarized the results of complement fixation in some 27,000 reported tests from the earlier literature and his own experience. The variety of antigens used included culture filtrates, Old Tuberculin, whole bacillary cells, partially defatted bacilli, lipoidal fractions, various watery extracts and polysaccharide. His analysis indicated that the isolated protein of the organism was not a satisfactory antigen for complement fixation and that the alcohol soluble substances were the best. More recent experience indicates that an antigen of partially defatted bacilli gives positive results in 60-70% of cases of active tuberculosis (22). It was anticipated that the chemical methods employed in the earlier studies may have yielded fractions of somewhat different antigenic properties from those obtained by more recent methods. In the present study therefore a wide variety of antigens was used in order to provide a general coverage of the types of antigenic material used by others in the past as well as to provide certain of the more striking chemical components of the organism in high concentration. All antigens were prepared from the H<sub>37</sub> strain of tubercle bacilli. Except where otherwise indicated the bacilli were cultured on a synthetic medium of the Long-Seibert type containing asparagine.

*Heat-killed bacilli.* Organisms cultured on synthetic medium over a period of a month were washed free of medium, ground in a mortar, and suspended in isotonic salt solution in a concentration of 10 mgs per ml. This suspension was heated at 65°C for one-half hour and was preserved by the addition of Zephiran (1:10,000). Dilutions of 1:50 or 1:100 of this suspension were employed in 0.10 ml amounts in the tests.

*Defatted bacilli.* Bacilli were partially defatted in accordance with

the method of Kolmer (22) A four week culture in glycerol-veal broth was washed with distilled water, dried over sulphuric acid, and ground to a powder in a mortar Each gram of bacillary powder was extracted with 200 ml of ether followed by 200 ml of acetone and then 200 ml of absolute alcohol In each case boiling was continued for one hour on an electric heater with a Liebig condenser All the organic extracts were discarded The dried bacillary residue was made up in a concentration of 1 gm per 200 ml of water This was boiled for one hour and then made isotonic by the addition of sodium chloride Phenol was added to a concentration of 0.25% as a preservative

*Culture medium filtrate* This antigen was the whole filtrate of a glycerol-veal broth culture prepared according to the technique of Petroff (17) The culture was filtered through several layers of paper, the filtrate was heated at 65°C for one-half hour and was then placed in sealed vaccine bottles which were heated at 60°C for thirty minutes

*Culture medium protein* Cultures were grown for eight weeks on synthetic medium Protein was prepared from the culture fluid by the method of Seibert and Glenn (23) without the use of heat The protein was precipitated by half saturation with ammonium sulphate at pH 7 and was reprecipitated eight times The final product was lyophilized and freshly prepared solutions in M/15 phosphate buffer of pH 7.3 were used for each set of tests

*Bacillary protein* Cultures were grown on synthetic medium The bacilli were washed, dried, ground and defatted as described above Protein was extracted with phosphate buffer (pH 7.3) and purified by reprecipitation several times from half saturated ammonium sulphate at pH 7 The purified protein was lyophilized and freshly prepared solutions in M/15 phosphate buffer of pH 7.3 were used for each set of tests

*Phosphatide and Wax* These lipids were prepared from bacilli grown on synthetic medium according to the methods of Anderson, as described in a previous publication (24) The phosphatide was chilled and emulsified in distilled water by grinding in a mortar The wax was emulsified by first dissolving it in chloroform and alcohol and adding to this an equal volume of salt solution, and subsequently evaporating the solvents over a water bath (25)

*Carbohydrate* This was obtained from the filtrates of cultures on

synthetic medium by the method of Masucci, McAlpine, and Glenn (26)

The antigens were employed in amounts determined in each case by preliminary anti-complementary tests. Of the whole killed bacilli, 0.1 or 0.2 mg. was used as the test does for each tube. Of the defatted bacilli, 0.1 mg. was used. The culture medium filtrate was used in 1:5 dilution. The culture medium and bacillary protein were used in 0.025 mg. amounts. Phosphatide was used in 0.05 mg., and wax in 0.05 mg. quantities. The carbohydrate was used in varying dilutions from 0.1 to 0.002 mg. All antigen doses were contained in 0.1 ml. volumes.

*Complement fixation tests.* The technique of carrying out these tests has been described in a previous publication (24). Particular attention was paid to the anti-complementary properties of each of the antigens and in every case titrations for this activity were set up in the presence of diminishing quantities of complement along with each set of tests. Each serum was run in duplicate, one set without antigen, so that the anti-complementary activities of the individual sera could also be appraised. The tests were incubated at 4°C. for 18 hours. Serum dilutions were used in amounts of 0.1 ml. and the same quantity of each antigen was employed with all serum dilutions. The end point reading was made in the last tube showing 50% hemolysis.

## RESULTS

*Comparison of antigens.* The efficacy of each of the antigens employed is indicated in Table 2 in which the results with tuberculous patients and with tuberculin-positive students are combined. The culture medium protein gave the greatest number of positive reactions of all the isolated derivatives of the bacillus tested. This has been found equally true in tests on experimentally infected animals (19). The similarly good result with culture medium filtrate was in all probability due to its high content of tuberculo-protein. The bacillary suspensions have been distinctly inferior to the protein solutions except in cases of active tuberculosis (See Table 4). Reactions with the phospholipid and wax have been too infrequent to be of any value. Tests with the carbohydrate have been uniformly negative.

The superiority of the protein antigens in these complement-fixation reactions was further indicated by the fact that in only one instance

was a positive reaction obtained with another type of antigen when the reactions with the protein antigens were negative. This exception was in the case of a tuberculin-positive student whose serum reacted to a dilution of 1:20 with the heat-killed bacilli but failed to react to defatted bacilli, culture medium filtrate, phosphatide or culture medium protein. In no instance did the bacillary protein give a positive reaction when the culture medium protein was negative among 14 cases in which the same serum was tested with both these antigens. In only one instance out of 35 tests did the culture medium filtrate give a positive reaction (in dilution of 1:20) when the culture medium protein was negative.

TABLE 2

*Combined results of complement fixation tests in patients with active tuberculosis and in healthy tuberculin-positive subjects*

TYPE OF ANTIGEN	NO. CASES TESTED	NO. CASES POSITIVE	PERCENT POSITIVE
Heat killed bacilli	41	5	12.2
Defatted bacilli	41	8	19.5
Culture medium filtrate	41	14	34.2
Culture medium protein	50	22	44.0
Bacillary protein	22	3	13.6
Phosphatide	41	3	7.3
Wax	26	2	7.7
Carbohydrate	28	0	0

In view of these facts it was considered warranted to use the culture medium protein alone in canvassing the sera during the last half of the experiment.

*Results in sarcoidosis* The complete data on the tests in cases of sarcoidosis are shown in Table 3. Those tests in which the sera were anticomplementary have been omitted. Six of the twenty-two cases gave positive reactions on one occasion. In only two of these cases was it possible to obtain a retest at a later date. In neither of them was a positive test obtained on the second or third sample of serum. In five of the six positive cases, positive reactions were obtained with more than one antigen. The superiority of the protein antigens is not as clearly demonstrated in this group as it was in the group of tuberculous subjects, probably because there were so few positive sera. In

TABLE 3

*Complement fixation reactions in 22 cases of sarcoidosis with antigens of M. tuberculosis*

CASE NO	DATE	HEAT KILLED BACILLI	DEF FATTED BACILLI	CULTURE MEDIUM FILTRATE	CULTURE MEDIUM PROTEIN	BACIL LARY PROTEIN	PHOSPHA TIDE	WAX	CARBOHY DRATE
1	7-21-44	0	0	0	0	0	0	0	0
2	8 17-44 12-13 46 9-16 47	2	4	4	0 0	0	4		0
3	10 19 44 4- 5 45	0 0	0 0	0 0	0 0	0	0 0	0 0	0
4	10 24-44	0	0	0	0	0	0	0	0
5	12- 4 44	1	1	2			0		
6	12-18 44 3-27-45	0 0	0 0	0 0	0	0	0 0	0 0	0
7	3 10 45	0	0	0	0		0	0	
8	3 20 45	0	0	0			0	0	
9	5 28 45 8 11 45	0	0	0	4 0		0	0	
10	6-12 45	0	0	1	2		0	0	
11	6 12 45	0	0	2	2		8	0	
12	6 12 45	0	0	4	2		2	0	0
13	9 17-45 9-19 45				0 0				
14	1-31 46				0				
15	12 13 46 1 21 47				0 0				
16	2 25 47				0				
17	3 3 47 7-17-47				0 0				

TABLE 3—*Continued*

ASE NO	DATE	HEAT KILLED BACILLI	DE FATTED BACILLI	CULTURE MEDIUM FILTRATE	CULTURE MEDIUM PROTEIN	BACIL LARY PROTEIN	PHOSPHA TIDE	WAX	CARBOHY DRATE
18	5-27-47				0				
19	6-6-47				0				
	7-23-47				0				
	9-18-47				0				
20	6-20-47				0				
21	8-20-47				0				
	9-18-47				0				
22	12-18-47				0				

The figures indicate dilutions of serum used in 0.1 ml. amounts

no instance did any other type of antigen give a positive result when the soluble protein antigens failed to do so

*Comparison of sarcoidosis with other groups* The cases of sarcoidosis have been compared in Table 4 with each of the other groups. For the sake of conciseness each individual antigen has not been listed separately. The antigens have been grouped into three types, viz., bacillary suspensions, protein solutions and lipid emulsions. This is justified by the close agreement between the results obtained with the sera of tuberculous patients with each antigen in the same group.

The last column of Table 4 summarizes the results in each group of cases with all the antigens used. It indicates a high proportion of positive reactions (61.7%) among cases with active tuberculosis. Healthy subjects with positive tuberculin tests gave a significantly smaller percentage of reactions (33.3%). There were no positive reactions among the control subjects who had negative tuberculin tests. The proportion of positive reactions in the cases of sarcoidosis (27.3%) was significantly lower than in the cases of active tuberculosis.

A comparison of the different groups of subjects in respect to their reactions with each type of antigen may also be made in Table 4. Only in the cases of active tuberculosis did the bacillary suspensions give an incidence of positive reactions comparable to those with the protein antigens. This was due to a high incidence of positive reactions



with the defatted bacillary antigen. Save for this one exception, the protein antigens gave more reactions than any other in all the groups.

*Titers of antibody* The complement fixation tests have been carried out with empirically determined optimal quantities of each antigen. Arbitrarily assigning a unitary value to this quantity of antigen, the unit of antibody may be defined as the amount reacting with a unit of antigen. The units of antibody per milliliter of serum may be calcu-

TABLE 4

*Comparison of complement fixation in cases of sarcoidosis, tuberculosis, and healthy subjects*

	TYPE OF ANTIGEN									ALL TYPES OF ANTIGEN	
	Bacillary* Suspensions			Protein† Solutions			Lipid‡ Emulsions				
	No tested	No positive	% positive	No tested	No positive	% positive	No tested	No positive	% positive	No tested	% positive
22 cases of sarcoidosis	13	2	15.4	22	6	27.3	12	3	25	22	27.3
26 cases of active tuberculosis	11	7	63.6	26	16	61.7	11	4	36.3	26	61.7
30 Students with positive tuberculin tests	29	3	10.3	30	9	29.7	29	1	6.7	30	33.3
29 Students with negative tuberculin tests	27	0	0	29	0	0	27	0	0	29	0

\* Includes the results with heat-killed bacilli and with defatted bacilli

† Includes the results with culture medium filtrate, culture medium protein and bacillary protein

‡ Includes the results with phospholipid and with wax

lated from the highest dilution of serum giving a positive reaction. On this basis a comparison of the positive sera from each of the groups is presented in Table 5.

There is a significant preponderance of higher titers in the cases of active tuberculosis in comparison with the other groups. This is most clearly shown with the protein antigens where the numbers of positive results were highest. Sixty-seven per cent of the positive sera of tuberculous patients had titers of 40 units per ml. or higher when tested with the protein antigens. Only thirty-eight per cent of the positive

sera of patients with sarcoidosis had titers of 40 units per ml with these antigens and none were higher. Twenty-three per cent of the positive sera of tuberculin positive students were 40 units per ml. A suggestive but less significant difference between the tuberculous and other sera is seen with the bacillary suspensions. There were too few positives with the lipid antigens to make a valid comparison.

TABLE 5  
*Distribution of titers of positive sera*

Units of antibody per cc of serum	TYPE OF ANTIGEN												
	Bacillary Suspensions				Protein					Lipid			
	10	20	40	80	10	20	40	80	160	10	20	40	80
6 Cases of Sarcoidosis	2	1	1		1	4	3				1	1	1
16 Cases of Tuberculosis	2	4	2	1	2	6	11	4	1	1	1	1	1
10 Students with Positive P P D	2	2			7	3	3			1			

#### COMMENT

Our experience indicates that complement-fixation reactions with these antigens of *M. tuberculosis* are specific for tuberculous infection. No positive reactions were obtained in a group of healthy individuals who had negative tuberculin skin tests and had negative histories and x-ray examinations. The results were not complicated by concurrent syphilis in which false positive complement fixation with tubercle bacillus antigens has been reported (22). The considerable number of negative tests on patients with active tuberculosis indicates, however, that detectable levels of circulating antibody may not always develop or persist during and after the infection.

There is no established relationship between skin sensitivity to tuberculin and the presence of circulating antibodies to the tubercle bacillus (9). Each develops in response to tuberculous infection and each may wane with the passage of time. The skin sensitivity develops with much greater regularity and usually persists for a longer time in

infected human beings. In the experimental animal, however, the skin sensitivity often disappears late in the course of infection while circulating antibodies to bacillary protein persist (19). The disappearance of skin reactivity has been observed in moribund patients with far advanced tuberculosis and also as a transient occurrence during the course of certain acute infections (9). In this connection one of the cases of active tuberculosis in the present study is of particular interest. This was a young negro woman with extensive pulmonary disease and a negative sputum who had a negative tuberculin test to 10 mg O.T. Her serum contained the highest titer of antibody (160 units per cc) of all the cases in this series. Shortly after the test she developed signs of tuberculous meningitis and eventually died. Autopsy verified the diagnosis of pulmonary and meningeal tuberculosis. This indicates that a high titer of antibody may be present in the serum of patients with active tuberculosis who have a negative tuberculin test just as in the experimental animals referred to above.

There is good general agreement among observers that a substantial majority of patients with sarcoidosis have a negative tuberculin test (2, 6, 8, 20). The tests on the present series of cases are in agreement with general experience. Those who adhere to the theory of tuberculous etiology of sarcoidosis have referred to this fact as evidence of a "positive energy", implying that the patient is desensitized by the infecting organism or its products (27). Since there is no known effect of circulating antibodies on the state of tuberculin sensitivity, it might be anticipated that antibodies to the bacillus would still be present in the circulating blood of the sarcoid patient even in the absence of skin sensitivity, if the disease were indeed due to this agent. Such a state of affairs has been observed in the tuberculous patient referred to above and in the experimental animal. The results of this study indicate that such an anticipation is not realized. The patients with sarcoidosis infrequently have circulating antibodies demonstrable by complement-fixation tests and those who do have antibodies tend to have a lower titer than patients with active tuberculosis.

The question remains as to the significance of antibodies to *M. tuberculosis* in the fraction of sarcoid patients in whom they were found. The group of patients studied was drawn at random from the hospital dispensary and offices of private physicians. They may not be

considered as representative of any limited social or economic group nor of the community as a whole. It would be fruitless to try accurately to predict the expected incidence of tuberculous infection among them. Since there is evidence that the incidence of positive tuberculin tests among cases of sarcoidosis is lower than it is among the population at large (2, 6, 8, 20), it is very likely that the incidence of past tuberculous infection among cases of sarcoidosis is higher than the incidence of tuberculin sensitivity, regardless of the etiology of this disease. The fact that about a quarter of the cases were sensitive to tuberculin suggests, therefore, that a somewhat larger proportion had had a past tuberculous infection. The presence of circulating antibodies to the tubercle bacillus in about a quarter of the cases is further evidence in favor of this deduction since only a third of the previously infected healthy subjects had antibodies. Altogether 8 of the 22 cases (36.4%) had *either* a positive skin test *or* positive complement-fixation. This suggests that a majority of them may have had a past tuberculous infection, a not unreasonable possibility in an urban group of this age distribution.

The etiological relationship of tuberculous infection to sarcoidosis, on the other hand, receives no support from this study. There is a large difference in the incidence of occurrence and in the titer of complement-fixation antibodies between the group of sarcoid patients studied and the patients with active tuberculosis. All the sarcoid patients had been biopsied relatively recently before the serological tests were done. In each case, the histological lesions were rich in epithelioid-cell tubercles. This is a feature which is a reliable indication of activity in tuberculous lesions. Moreover sarcoidosis is known to be a chronic, slowly progressive disease with evidence in many instances of continuous or recurrent spread over months or years. The clinical symptoms and observations in most of these cases indicated that the disease had recently progressed or was currently doing so. Therefore in order to show convincingly by serological means that the disease is of tuberculous etiology, it would be necessary to demonstrate a serological pattern that closely resembled that of active tuberculosis. This has not been done.

Unless patients with sarcoidosis have an impaired ability to produce or maintain circulating antibodies, the data presented here constitute

strong evidence against the tuberculous etiology of this disease. In view of the indications that patients with sarcoidosis suffer some disability in manifesting hypersensitivity to tuberculin following vaccination with avirulent bacilli (11, 12, 13, 28), it might reasonably be questioned whether they have the capacity to develop antibodies to the bacillus when infected as readily as other individuals (29). An attempt is being made at present to determine whether any difference exists in the ability of patients with sarcoidosis to develop antibodies to *M. tuberculosis* when vaccinated with avirulent bacilli in comparison with healthy individuals.

#### SUMMARY

Complement fixation reactions have been performed on the sera of twenty-two cases of sarcoidosis, using a variety of antigens prepared from the H37 strain of *Mycobacterium tuberculosis*. There was no clinical or pathological evidence of active tuberculosis among these patients and only four of them had positive tuberculin skin tests. Positive serological reactions were obtained on five (27.3%) of the cases. In contrast to this, sixteen (61.7%) of twenty-six cases of active tuberculosis gave positive serological reactions and the average titer was higher in this group than it was among the cases of sarcoidosis. Ten (33.3%) of thirty healthy control subjects, who had positive tuberculin tests, gave positive complement fixation reactions with a range of titers similar to that of the cases of sarcoidosis. It is believed, therefore, that the positive complement fixations observed among the cases of sarcoidosis may be due to a past tuberculous infection unrelated to the sarcoidosis. Unless patients with sarcoidosis have an impaired ability to develop complement fixing antibodies to the tubercle bacillus, the considerable difference in serological reactions between them and cases of active tuberculosis indicates a difference in the etiology of these diseases.

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## CLINICAL THERAPEUTIC TRIAL OF A NEW DRUG<sup>1</sup>

The large number of new drugs which have been put forward in recent years as agents in the treatment of disease has raised many problems, none of which is more difficult of solution than the decision concerning their safety and efficacy in the treatment of the diseased patient. The conference today offers pharmacological and statistical advice on the design of therapeutic trials to the clinician who must ultimately be responsible for these decisions. The discussion will be opened by Dr. E. K. Marshall, Jr.

Dr. E. K. Marshall, Jr. I should like to outline what data on the pharmacology of a new drug are, in my opinion, essential, and what are desirable before the drug is submitted to clinical therapeutic trial. First, we have the question of the safety of the drug for the patient. A great deal, but by no means everything, about this safety factor can be learned from carefully planned experiments on the toxicity of the drug for animals. If the drug is found to exhibit definite signs of disturbance of the function of various organs, such as kidney, liver, hematopoietic or nervous system, it is extremely likely that sufficient dosage will cause the same type of toxic injury in man. On the other hand, where no toxic symptoms have been observed in animals except with very large doses of the drug, certain types of toxic symptoms may occur in man from small doses. These are mainly of the nature of allergic reactions, skin reactions and blood dyscrasias. These toxicity experiments should be done with a dosage schedule of drug similar to that which one expects to employ in the therapeutic use of the drug in man. A determination of the acute toxicity of the drug in animals (the toxicity resulting from a single dose) may give no information of value where one expects to give more than one dose of drug a day for several days in the clinical trials. Thus, quinine is much more toxic than quinacrine when a single dose is given, but the reverse is true when the drugs are administered several times a day over a period of several days. Also, it is important to determine toxicity on more than one species of animal. The therapeutic dose of quinine for man based on concentrations of drug in the plasma causes blindness in the dog.

<sup>1</sup> Therapeutic Conference held April 30, 1949



after a few days administration. In the monkey as in man this dose or multiples of it can be given without causing blindness or any serious toxic symptoms. An investigation of the toxicity of the 8-aminoquinolines (the only drugs known to be curative of vivax malaria) showed that in the case of these extremely toxic drugs data obtained in the monkey, but not those obtained in the dog, rabbit, rat or mouse, can be carried over to man. If possible, and particularly with extremely toxic drugs, both the monkey and dog should be included in the species used for toxicity studies. The doses of drug given to animals should include those large enough to cause symptoms, so that one may get some idea as to what to look for when the drug is given to man. Of great value in all these toxicity studies is the use of another drug of the same general chemical type, which has been given to man, as a standard of comparison.

While not essential, it is desirable to have some knowledge of the absorption, excretion, distribution, and fate of the drug before clinical trials are begun. This kind of information allows one to arrange dosage schedules in relation to the presence of drug in the organism and to study possible cumulation of the drug.

It is, of course, necessary to have a definite idea of the purpose for which the drug is to be given a clinical trial. The possible therapeutic value of the drug is obtained from animal experiments. These will be of many different types depending on the particular pharmacological actions of the drug. A potential chemotherapeutic agent is tested against some experimental infection in animals. If possible, this infection should be produced with the same species of parasite which causes human disease. If the drug under consideration is not a potential chemotherapeutic agent, the pharmacological studies may be of a variety of types in order to assess the possible therapeutic value of the drug. Since it is very difficult to conduct adequately controlled therapeutic studies on man, it is important to assess the therapeutic value of the drug in animals as carefully as possible as a basis for its therapeutic use. Here, again, a drug of similar nature already used in therapy should be used as a standard of reference for the new drug.

After animal experiments have been completed, the data obtained are checked by performing pharmacological studies on man. These are aimed mainly to confirm data on the absorption, excretion, dis-

tribution and degradation of the drug obtained in animals, and to assess in a preliminary way the toxicity of the drug for man. Since, many toxic reactions which are not seen in animals may occur in man, great care should be used in the initial administration of the drug to man. Somewhere around one-fifth or one-tenth of the dose which would appear from animal experiments to be safe in man might be given as a start. Only a single dose should be given initially, as unexpected untoward reactions are less likely with this procedure than with multiple doses. The drug is now ready for assessment as to its therapeutic value. The conduct of human therapeutic experiments with proper controls is by no means simple. The discussion of this phase of the subject, I shall leave to Dr. Merrell.

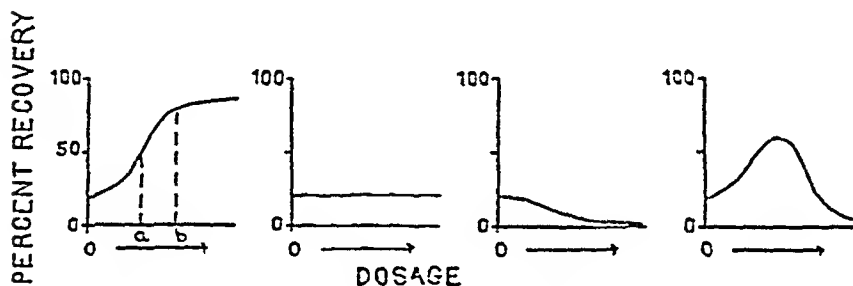


FIG 1

FIG 2

FIG 3

FIG 4

FIGS 1-4

Dr. Margaret Merrell. The problem of human therapeutic tests on a drug is that of measuring some defined reaction or reactions, favorable or unfavorable, against varying doses of the drug. I shall discuss the issues first around the example of recovery from a specific disease.

We may visualize various patterns of dosage and percentage recovery. Some of these are illustrated in the following figures.

Fig. 1 shows the situation where about 20% recover without the treatment but the percentage increases to about 80% with increasing dosage. It never reaches 100% and beyond dose *b* there is no appreciable gain with increasing dosage. This is the sort of pattern we usually visualize with a drug that has definite therapeutic value.

It is well to keep in mind that quite different patterns may represent the actual situation. Fig. 2 is the case of a drug that does not affect

the chances of recovery at all, fig 3 shows the situation for the drug that affects survivorship unfavorably, and fig 4 indicates a drug which has a therapeutic value up to a point, but beyond that it is unfavorable, that is, there is an optimum dose

The first experiment in evaluating a new drug clinically may well be the test of the drug at some fixed dose against a zero dose. The hope is that we will be dealing with the situation in fig 1 and it is desired to take a dose which is large enough (say dose *a*) to permit telling whether we are dealing with the case of fig 1 or fig 2 but not large enough to be dangerous should we actually be dealing with the cases of figs 3 or 4. We would anticipate that if we get favorable results, then we would go on to a further examination of the dosage scale.

I shall confine my discussion to this first experiment (dose *a* against dose zero) since the issues I want to consider are the same for this experiment as for the further ones involving varying dosages. These issues are 1) comparability of the groups entering the test, and 2) comparability of the measures of the effect.

### *Setting up a Control*

The first issue is that of getting a control, that is having the two groups receiving dose *a* and dose zero similar initially and in the course of treatment, for all things except the dose itself. Similarity refers to those things that affect the prognosis. However, it is impossible to identify all the elements that affect the outcome and therefore we require some process that will bring about similarity in our groups without attempting to categorize all the important factors and match the groups on those. We may do no matching at all, or we may specify certain characteristics for which we match the groups, such as sex, color, age etc, but within these classes we must have comparability for the numerous unspecified factors which may be influential.

This is achieved by some sort of randomizing process for assigning patients to the two groups, such as even and odd history numbers, or persons born in even and odd months of the year, or an assignment by lot. For this process to be effective, it is necessary to have a reasonably large number in each group, since we are counting on chance to achieve comparability in our groups. The question as to what is "reasonably large" depends upon the problem and the factors involved, but it is a rare problem indeed where a series of less than 20 per group is adequate.

and often much larger numbers are needed. It is worth emphasizing that it is better to build up a well-controlled series in this way even if it has to be done gradually than to get a larger series poorly controlled. Large numbers in themselves are worse than useless if the groups are not comparable, since they encourage confidence in an erroneous opinion.

After the two groups have been set up it is well to compare them for various attributes to see that they are in fact similar for these factors. This constitutes a check on the procedure used. We must keep in mind that the object of the effort to achieve similar groups is that whatever difference we find in our results may be attributed to the drug and not to the fact that the groups were dissimilar in the first place.

It may be well to mention two or three types of "controls" that are not satisfactory. One is the historical control, for example the observation that in the pre-drug days, only  $m$  per cent reacted favorably and with the drug in use this is changed to  $n$  per cent. If everything remained constant in the situation, this would be all right, but there is no assurance that this is the case. There are differences in treatment aside from the specific drug, difference in type of patients presenting themselves, often due to the very fact that a drug is available, difference in the general economic and health set-up of the patients. A second unsatisfactory control is that made up of patients who refuse the treatment. One can think off hand of many ways in which such patients may differ from those accepting treatment. The impossibility of assessing the importance of these differences means that the results are incapable of interpretation and therefore the observations are wasted. Another control involving the same difficulties of interpretation is that obtained from another clinic not using the treatment.

In presenting the data on a clinical experiment the actual procedure used in selecting cases and assigning them to the two groups should be explicitly defined. If the study has been well-conducted, rules must have been laid down and these should be stated. It is difficult to evaluate the work reported in a paper when the description is given in such vague phrases as "consecutive cases." The absence of a more specific description leads naturally to a doubt as to the author's awareness of the difficulties surrounding the setting up of a good control and consequent doubt of the interpretation.

Once comparable groups have been established it is necessary that

they should be treated in exactly the same way. It is of course for this reason that a placebo or some other drug is used to control various environmental and psychological factors that may influence the outcome. These influences may be very subtle but nevertheless potent and it is desirable whenever possible to have the person administering the treatment unaware of the identity of the drugs.

### *Measurement of Effects*

The effects on which the drug is to be assessed should be defined at the outset and not left to be ferreted out when the results are in. Definitions and objective criteria should be laid down which will be alike for the two groups. If the effect is death or recovery this is easy, but even here certain criteria must be specified, such as the time cut-off of deaths to be counted, since the longer the patients are followed the more of them will die. The definition "death in hospital" is unsatisfactory because length of hospitalization may be a factor of consequence. It is important also to define any exceptions that may be made on cause of death, since exclusion of "non-specific" deaths may allow bias to creep in.

If the measurements are on x-ray findings, lesions, skin reactions, etc., it is important to make the criteria as objective as possible. However it is impossible in such measurements to rule out some subjective judgment on borderline cases. It is with this difficulty in mind that it is recommended that the person making the judgment have no knowledge of the treatment group to which the patient was allocated. The difficulty of avoiding unconscious bias is so great that the experimenter is in a peculiarly advantageous position if he can make his judgments unburdened by knowledge of the patient's treatment.

### *Illustrations*

Numerous examples of the importance of these various features of a control could be cited from the medical literature. I want to mention certain features of three well-controlled clinical experiments by way of illustration. The first concerns the value of gelatin as an energizer. Uncertainty concerning its merit persisted for some years because of a number of inadequately controlled tests reported in the literature.

An interesting experiment was reported in 1942 (1) in which 33 volunteers from the football teams in an institution formed the test and control groups. The description of the experiment is in part as follows: "Two sets of tablets similar in appearance, shape, form, and taste were specially prepared by a pharmaceutical house. The investigators knew only that one set contained aminoacetic acid while the other set was a mixture of lactose and saccharose. The identity of the tablets was not revealed until the experiment had been completed, the final calculations made and conclusions drawn." The instrument used to measure work was a bicycle ergometer constructed so that work output could be translated into watt output per second. For 13 days there was a preliminary training and study period and then the tablets were given four times daily, with the explanation to the subjects that a test was being made to find out whether gelatin increased work capacity. The men were led to believe that all the tablets contained gelatin.

Figs. 5 and 6 show the results of the experiment. It will be seen that during the preliminary test period the work output was virtually level, but that both groups showed a marked increase while receiving the capsules. After 34 days the capsules were switched, so that the men who had been given the gelatin now received the placebo and vice versa. By this time the men had about fulfilled their potentialities and both groups levelled off. If the placebo had not been a part of this experiment, but instead, a "before and after" comparison had been made, it is almost inevitable that the conclusion would have been drawn that aminoacetic acid was an effective energizer. The authors emphasize the importance of the experimental design in the conclusion that "a study of the effects of a drug on fatigue must be meticulously controlled: the drug under study should be administered alternately with a placebo identical in physical characteristics, and the identity of each should be unknown to the subjects as well as to the investigators." From a wide variety of studies in other fields, it may be said that this statement holds generally and is by no means limited to studies on fatigue, where admittedly the psychological factor is an important one.

The second example is one of the war-time sea trials to assess the relative value of various motion sickness preventives. Two drugs and

a placebo were under test. For administrative reasons it would have been easier to use a single remedy on a given boat. It was argued that the boats were alike and were to run parallel courses. However, the persons designing the experiment insisted that all three remedies should be used on each boat and this was done. When the results were in, it was found that the men on one boat had lower rates for each of

*Work Output Associated with the Administration of Aminoacetic Acid*

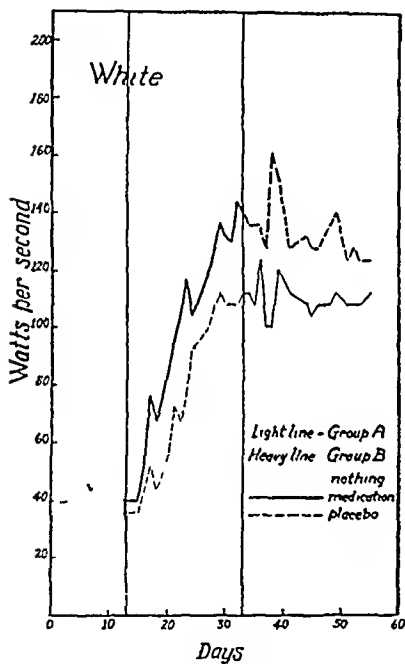


FIG 5

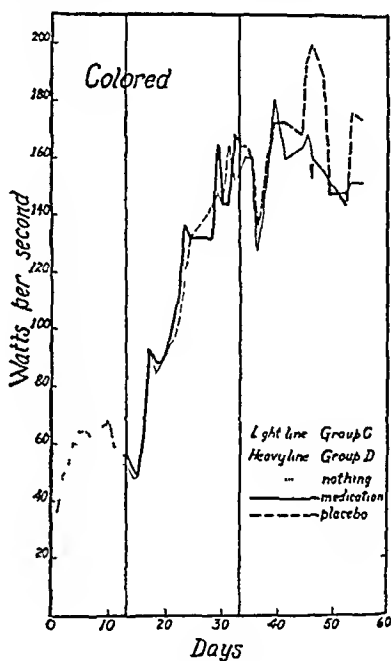


FIG 6

the three remedies than the men on corresponding remedies on the other boat. Investigation revealed the fact that there was a difference in the ballast this boat was carrying. This was a fact which was quite inapparent to the experimenters and they would never have bothered to look for it if the boat had contained a single remedy. Had this been the case it would naturally have been inferred that this remedy was particularly efficacious, since the boats and courses run were alike.

Finally I want to cite a clinical experiment conducted by the

Medical Research Council on streptomycin treatment of pulmonary tuberculosis (2) The report merits study not only for the results but for the way the experiment was conducted The first part of the report takes up a careful definition of the type of case accepted for study, how the cases were obtained, and how they were allotted to the streptomycin and control groups "Determination of whether a patient would be treated by streptomycin and bed-rest (S case) or by bed-rest alone (C case) was made by reference to a statistical series based on random sampling numbers drawn up for each sex at each centre by Professor Bradford Hill, the details of the series were unknown to any of the investigators or to the coordinator and were contained in a set of sealed envelopes, each bearing on the outside only the name of the hospital and a number After acceptance of a patient by the panel, and before admission to the streptomycin centre, the appropriate numbered envelope was opened at the central office, the card inside told if the patient was to be an S or C case, and this information was then given to the medical officer of the centre "

I have cited just one feature in the design of this study—that of assignment of patients to the two groups The same scrupulous planning applied to other features of the study, and finally to the presentation of results It is possible to find out from the report just what was done, and what the outcome was for patients falling into various diagnostic classes The result is that a rather limited number of cases, only 107 all told, have served to give definitive results which one can interpret with confidence

### *Statistical Aid*

In dealing with clinical studies of this type, it is not at all uncommon for the clinician to seek statistical aid after the results are in, to find out whether differences are significant, or in some other way to have assistance in the analysis If such a move is contemplated it is well to seek this assistance at the outset for there are no statistical maneuvers that will rescue a study that has not been well controlled Statistical constants can be computed and it can be determined whether a difference is significant, but whether or not the difference is associated with the therapy or with the unknown or unthought of factor, such as ballast in the ship, is anybody's guess



*Practical Problems*

Some of the recommendations and requirements listed above for a human experiment of this type may appear impossible of achievement. The question of the doctor's duty to the patient and the demands of the patient for a particular treatment immediately come to mind. Many of the potential difficulties do not arise, however, if the clinical experiment can be carried out very promptly following the necessary pharmacological tests. By "promptly" I mean as soon as the drug is ready to be tried on patients at all. The very first trials should be in terms of a well-controlled experiment.

It is easy to fall into the error of assuming that a new drug must be good and therefore it is unethical to withhold it from a patient. It must be remembered, however, that we may be dealing with the situation in fig. 3. It may well be that those from whom the drug is withheld are in fact the fortunate ones, and that by not setting up a control this fact is not immediately discovered.

Another reason for prompt action is that a good clinical experiment is far easier to achieve before the word of the new antibiotic reaches the news stands. And if it has, unfortunately, received early publicity, the drug may be relatively scarce at the outset, a fact that may be used to advantage.

If advance plans for the type of human experiment required are made while the pharmacologist is still carrying on the necessary laboratory work then, when this is complete, controlled trials can begin immediately and many of the hurdles which a delay is likely to erect, will not be there to obstruct the work.

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# STUDIES ON THE INITIATION OF BLOOD COAGULATION

## I THE RELATIONSHIP OF PLATELETS TO THE CLOT-PROMOTING EFFECT OF GLASS SURFACES<sup>1</sup>

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Whole blood appears to contain all of the substances which are necessary for spontaneous coagulation. Blood obtained by techniques designed to prevent contamination with tissue juice or other extraneous material will clot spontaneously in a glass receptacle. In the classical theories of blood coagulation the initiation of clotting is attributed to the presence of thromboplastin, a substance which has the property of converting prothrombin to thrombin. An appreciable amount of effective thromboplastin is apparently not available in blood *in vivo* since intravascular clotting does not normally occur. The absence of effective thromboplastic activity from freshly drawn normal blood is further suggested by the great prolongation of the clotting time which is observed when blood is permitted to come in contact only with oiled or paraffined surfaces. Apparently on contact with certain foreign surfaces blood may undergo a change in which effective thromboplastic activity appears. The present studies are concerned with the nature of the alteration which takes place in blood on contact with glass surfaces.

According to the classical theory of blood coagulation the source of thromboplastin in blood is believed to be the platelets (1). While this belief has been widely accepted, some investigators have presented evidence suggesting that platelets are not necessary for the *initiation* of clotting (2, 3, 4, 5, 6, 7, 8). These workers believe that all of the components of blood required for spontaneous coagulation are present in platelet-free plasma. The use of silicone in blood clotting studies (9) has greatly facilitated the investigation of this problem. Nevertheless,

<sup>1</sup> This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U S Public Health Service.

results obtained with this technique have been conflicting (8, 10, 11), and there is no agreement concerning the rôle of the platelets in the initiation of coagulation

Previous studies have shown that when normal human plasma is rendered severely platelet-deficient, its clotting time in silicone-treated tubes is significantly longer than in glass tubes (8). Not infrequently essentially platelet-free plasma does not clot spontaneously in silicone-treated tubes although the same plasma clots readily in glass tubes. Contact of platelet-deficient plasma with a glass surface apparently results in an alteration which may not occur on contact with a silicone-treated surface. There are two possibilities concerning the manner in which glass surfaces serve to promote coagulation of essentially platelet-free plasma: (1) a few platelets remain and are essential for the initiation of clotting, or (2) the clotting process occurs independently of platelets. In the experiments to be described an attempt has been made to shed further light on this problem by observing the effects on coagulation of varying independently the concentration of platelets and the area of glass surface to which normal human plasma is exposed.

#### METHODS AND MATERIALS

Essentially platelet-free plasma was prepared by high-speed centrifugation of human blood at low temperatures, using silicone-treated<sup>2</sup> apparatus as previously described (8). No anticoagulant was employed. In most of these experiments the silicone-treated surfaces were reinforced by a film of silicone oil.<sup>3</sup> During the preparation of the plasma its temperature was not permitted to rise above 2°C. Special precautions were taken during the centrifugation period to ensure that the temperature of the plasma in the centrifuge cups did not exceed 2°C. Freezing of the plasma was not permitted to occur. Platelet-rich plasma was obtained in the same manner by low-speed centrifugation. Plasmas with different platelet concentrations were prepared by mixing platelet-deficient and platelet-rich plasmas from the same source. Platelet counts on platelet-rich plasma were performed using Rees-Ecker dilu-

<sup>2</sup> General Electric Dri-Film 9987

<sup>3</sup> Silicone oil 9996-200 centistokes was kindly supplied by Dr E. G. Rochow, Department of Chemistry, Harvard University

ent, and on platelet-deficient plasma by direct microscopic observation of the undiluted plasma as previously described (8)

Clotting times of 1 ml portions of plasma were determined at 37°C in scrupulously clean Pyrex test tubes (13 x 100 mm) using in most instances three tubes for each determination. The tubes were observed for a minimum of 24 hours.

Prothrombin times were determined by the one stage method of Quick using rabbit brain thromboplastin prepared according to the technique of Brambel (12). Recalcified plasma clotting times were measured at 37°C following the addition of 0.3 ml of M/40 calcium chloride solution to a mixture of 0.3 ml each of decalcified plasma and 0.85% sodium chloride solution. Decalcified plasmas were prepared by the addition of 10% by volume of M/10 sodium oxalate to native plasmas obtained by the silicone technique previously described. The prothrombin activity remaining in the serum after clotting was estimated by determination of the prothrombin time of the serum. 0.1 ml of serum was added to a mixture of 0.1 ml of rabbit-brain thromboplastin and 0.1 ml of recalcified prothrombin-free plasma prepared by barium sulfate adsorption. The time required for clot formation at 37°C was then noted.<sup>4</sup>

Suspensions of macerated platelets were prepared from the sediment obtained by high-speed centrifugation of platelet-rich plasma. This sediment, which was composed of a solid, tenacious mass of platelets, was washed 6 times with 0.85% sodium chloride solution and then ground with a glass mortar and pestle. There appeared to be no loss of thromboplastic activity as a result of the repeated washings and maceration. From the platelet count of the original plasma and the volume of the saline in which the macerated platelets were finally suspended, the "platelet equivalent" of the suspension was estimated. Microscopic observation revealed that few if any intact platelets remained.

Crushed glass was prepared from Pyrex test tubes of the same lot used for clotting time determinations. Particles which passed through

<sup>4</sup> This procedure is not an accurate measure of residual prothrombin since the result may be influenced by other serum constituents. However, when thrombin is absent and changes in prothrombin are large, this technique seems to provide a satisfactory index of the amount of prothrombin disappearing during clotting.

a 32 mesh screen were treated with chromic acid, rinsed with copious quantities of distilled water, and dried in an oven

# RESULTS

## *The Clotting Time of Platelet-Deficient Plasma in Glass and Silicone-Treated Tubes*

When human plasma was rendered severely platelet-deficient there was a marked difference between its clotting times in glass and in sil-

TABLE I

*Clotting Times of Severely Platelet-Deficient Plasmas in Glass and Silicone Treated Tubes*

EXPERIMENT	NATIVE PLATELET DEFICIENT PLASMA		
	Platelets	Clotting time of 1 ml portions at 37°-C	
		Glass tubes	Silicone tubes
	<i>per cmm</i>	<i>min</i>	<i>min</i>
I	1	17	No clots
II	1	70	No clots
III	2	23	No clots
IV	3	49	No clots
V	3	54	No clots
VI	4	42	No clots
VII	6	97	No clots
VIII	10	39	No clots
IX	50	30	No clots
X	110	21	No clots

Plasmas were observed for clot formation for at least 24 hours

cone-treated tubes In Table I the data of 10 experiments are presented in which essentially platelet-free plasma showed no evidence of clotting in silicone-treated tubes at 37°C during a period of observation of at least 24 hours In every instance, however, relatively prompt clotting occurred in glass tubes The fact that these plasmas did not clot in silicone-treated tubes provides definitive evidence that no thrombin or effective thromboplastin was present in the plasma at the time of its introduction into glass tubes It is apparent from the data in the table that there was no relationship between the clotting time in glass tubes and the number of platelets remaining

TABLE II

*Effect of the Addition of Crushed Glass on the Clotting Time of Plasma with Varying Platelet Concentrations*

## Experiment I

	PLATELETS (PER CMM)			
	8	167 500	385,000	670 000
Clotting time (min )	4	4	4	3
	4 5	4	4	3 5
	5 5	4	4	4

## Experiment II

	PLATELETS (PER CMM)			
	1	52 500	105 000	210 000
Clotting time (min )	6	7	7	7
	7	8	7	8
	9	9	8	8

## Experiment III

	PLATELETS (PER CMM)			
	23	16 000	156 000	312 000
Clotting time (min )	4	4	4	4
	4	4	4	4
	4	4	4	4

Plasmas with different platelet concentrations were obtained by mixing platelet-deficient and platelet-rich plasma from the same source 150 mgm of crushed glass was added to 1 ml portions of plasma and the clotting time at 37°C determined in triplicate

When no crushed glass was added the clotting times in glass tubes were much longer in each instance

*Effect on the Rate of Clotting of Varying the Platelet Concentration when the Glass Surface Area is Constant*

When the glass surface area was relatively limited, as in ordinary test tubes, the clotting time of plasma appeared to be inversely related to the platelet concentration, if changes in the latter were large (8) However, when the surface in contact with plasma was increased by the addition of crushed glass, the rate of clotting became essentially

independent of the platelet concentration over an extremely wide range (Table II) Under these conditions the most platelet-deficient plasmas obtained clotted in approximately the same time as those containing several hundred thousand platelets per cmm

TABLE III

*Effect of Crushed Glass on the Rate of Clotting of Platelet-Rich and Platelet Deficient Plasmas in Glass Tubes*

Experiment I—Effect of different amounts of crushed glass on the rate of clotting of platelet rich plasma (Platelets 410,000 per cmm)

	CRUSHED GLASS (MG/ML PLASMA)								
	0	10	25	50	100	150	300	500	800
Clotting time (min)	9	6	5.5	4	4.5	3	3	2.5	2.5
	9	6	6.5	5	4.5	3.5	3	2.5	2.5

Experiment II—Effect of different amounts of crushed glass on the rate of clotting of platelet deficient plasma (Platelets 16 per cmm)

	CRUSHED GLASS (MG/ML PLASMA)								
	0	10	25	50	100	150	300	500	800
Clotting time (min)	17	10	8	7.5	7	7	6.5	6	5
	40	11	9.5	9	9.5	7	7.5	6.5	6

Experiment III—The relationship of particle size to the clot promoting effect of crushed glass on platelet deficient plasma (Platelets 6 per cmm)

	CRUSHED GLASS 150 MG/ML PLASMA				
	>16 Mesh	16 Mesh	32 Mesh	50 Mesh	100 Mesh
Clotting time (min)	8	6	5.5	6	4
	9	7	6	3.5	3.5

The clotting times of 1 ml portions of plasma were determined in duplicate at 37°C

In experiments I, II and III the subjects as well as the lots of crushed glass were different, so that the results are not strictly comparable

*Effect on the Rate of Clotting of Varying the Area of Glass Surface When the Platelet Concentration is Constant*

The effect of adding varying amounts of crushed glass to platelet-rich and to platelet-deficient plasma is shown in Table III. With glass of the same particle size there was an inverse relationship between the amount of glass added and the clotting time, regardless of the platelet

concentration Relatively small amounts of crushed glass were very effective in shortening the clotting time of severely platelet-deficient plasma When a constant weight of crushed glass was added to plasma, the rate of clotting was inversely related to the glass particle size

TABLE IV  
*Clot Accelerating Effects of Macerated Platelets on Platelet-Deficient Plasmas*  
Experiment I—Original Platelet concentration of plasma 3 per cmm

	TYPE OF TUBE						
	Glass	Silicone	Glass	Glass	Silicone	Glass	Silicone
	Crushed glass (mgm)						
	0	0	150	0	0	0	0
Macerated platelets (final concentration per cmm of plasma)	0	0	0	200,000	200,000	2000	2000
Clotting time (min) at 37°C	10	20	6	8	11	18	19
	17	49	6.5	8	12	18	27
	26	120	—	—	—	31	55

Experiment II—Original platelet concentration of plasma 15 per cmm

	TYPE OF TUBE						
	Glass	Silicone	Glass	Glass	Silicone	Glass	Silicone
	Crushed glass (mgm)						
	0	0	150	0	0	0	0
Macerated platelets (final concentration per cmm of plasma)	0	0	0	90,000	90,000	900	900
Clotting time (min) at 37°C	12	15	6	7	—	7	19
	14	32	6	8	—	12	20
	19	51	6	8	—	16	51

0.1 ml of suspensions of macerated platelets were added to 0.9 ml of platelet-deficient plasma to give final concentrations of platelets recorded

The clot accelerating effect of the macerated platelets was compared to that of crushed glass

#### *Clot Accelerating Effect of Macerated Platelets*

The effect of macerated platelets on the clotting time of platelet-deficient plasma is shown in Table IV. Concentrated platelet suspension was less effective in accelerating coagulation than was crushed



glass Furthermore, when crushed glass in adequate amounts was added to platelet-deficient plasma the clotting time was not further reduced by the simultaneous addition of macerated platelets Diluted platelet suspensions had relatively little effect on the clotting time of platelet-deficient plasma in glass and silicone-treated tubes In experiment I of Table IV, for example, the addition of sufficient platelet-material to provide a final concentration of 2000 macerated platelets per cmm, shortened the clotting time in silicone tubes only from 120 to 55 minutes On the other hand, addition of 150 mgm of crushed glass to this plasma containing only 3 platelets per cmm shortened the clotting time to approximately 6 minutes Concentrations of macerated platelets equivalent to many times the number of platelets originally present in centrifuged plasma failed to eliminate the difference between the clotting times in glass and silicone-treated tubes In view of these results, it seems improbable that the increased rate of clotting of essentially platelet-free plasma in glass as compared to silicone-treated tubes can be attributed to the presence of a few remaining platelets

*Effect of Incubation on the Reactivity of Platelet-Deficient Plasma to Glass Surfaces*

Platelet-deficient plasma which remained fluid in silicone-treated tubes at 37°C for 24 hours (Table I) occasionally clotted at the end of that time following the addition of crushed glass However, in most cases such stored plasma was not clotted by glass At times the reactivity of plasma to crushed glass was lost after only 6 hours incubation at 37°C When plasma had been stored for a sufficient length of time so that it was no longer clotted by ground glass, it could still be clotted by rabbit-brain thromboplastin Even when platelet-deficient plasma was stored at relatively low temperatures (2-7°C) for 24 hours, there was occasionally a loss of reactivity to glass surfaces at 37°C

*Relationship of Glass Surface Area and Platelet Concentration to the Amount of Prothrombin Converted During Clotting*

When plasma clotted in silicone-treated tubes, the amount of prothrombin remaining after clotting was relatively large, regardless of the platelet concentration (Table V) In glass tubes on the other hand, there was an inverse relationship between the number of platelets

present and the quantity of prothrombin remaining. At a given platelet concentration the amount of prothrombin which disappeared during clotting was related to the glass surface area (Table VI). Even when

TABLE V  
*Relationship of Platelet Concentration to the Rate of Conversion of Prothrombin*

		PLATELETS (PER CMM)						
		0	22 200	44 400	68 600	222 000	333 000	444 000
Residual prothrombin times (sec.)	Glass tubes	19	29	35	38	53	84	90
(4 hours after clotting)	Silicone tubes	18	21	23	23	27	26	24

The plasmas used in this experiment were derived from a single source, and allowed to clot at 37°C in glass and silicone-treated tubes. After 4 hours of incubation at 37°C the prothrombin times of the sera were determined as described in the section on methods.

TABLE VI  
*Relationship of the Glass Surface Area to the Rate of Conversion of Prothrombin*

TIME OF DETERMINATION IN RELATION TO CLOTTING	PROTHROMBIN TIME OF SERUM DERIVED FROM							
	Platelet rich plasma* allowed to clot in the presence of crushed glass (mgm crushed glass/ml of plasma)				Platelet-deficient plasma** allowed to clot in the presence of crushed glass (mgm crushed glass/ml of plasma)			
	0	150	500	800	0	150	500	800
	Sec	Sec	Sec	Sec	Sec	Sec	Sec	Sec
Before	16	—	—	—	16	—	—	—
2 hours after	18	111	122	100	—	—	—	—
4 hours after	34	215	297	600	22	24	29	37

\* Platelet-rich plasma contained 464,000 platelets per cmm

\*\* Platelet-deficient plasma contained 7 platelets per cmm

Plasma was allowed to clot at 37°C in the presence of the amount of crushed glass indicated.

After 2 and 4 hours of incubation at 37°C the prothrombin time of the serum was determined as described in the section on methods.

plasma was severely platelet-deficient, this relationship persisted. Maximal conversion of prothrombin occurred only when both the platelet and surface factors were adequate.

#### *Observations on the Mechanism of Action of Glass Surface in Coagulation*

Crushed glass failed to cause the coagulation of oxalated plasma. The recalcified plasma clotting time was shortened after incubation

TABLE VII

*Effect on the Recalcified Clotting Time of Oxalated Plasma of Incubation in Tubes of Different Surfaces and with Crushed Glass*

	OXALATED PLATELET DEFICIENT PLASMA (9 PLATELETS PER cmm)						
	Incubated in glass tube		Incubated in tube with crushed glass 450 mgm per ml		Incubated in silicone treated tube		
	Recalcified in Glass tube		Recalcified in Glass tube		Recalcified in Glass tube		Recalcified in Silicone treated tube
Recalcified in presence of crushed glass (mgm)	0	150	0	150	0	150	0
Recalcified clotting time (min)	12 5	7	8	7	13	7	16
	13 5	7	8	7	16	7	17 5

Plasmas oxalated after the removal of the platelets were incubated at 37°C for 15 minutes with and without crushed glass. Following incubation the plasma was recalcified in glass and in silicone-treated tubes with and without added crushed glass.

TABLE VIII

*Effect of Crushed Glass on the Recalcified Plasma Clotting Time in the Presence of Highly Diluted Rabbit-Brain Thromboplastin*

Experiment I—Platelet rich plasma (platelets 240,000 per cmm)

	THROMBOPLASTIN CONCENTRATION							
	0		1 3200		1 800		1 200	
	Crushed glass (mgm/ml plasma)							
	0	150	0	150	0	150	0	150
Recalcified clotting time (sec )	205	96	130	88	72	58	46	39

Experiment II—Platelet deficient plasma (platelets 35 per cmm)

	THROMBOPLASTIN CONCENTRATION							
	0		1 3200		1 800		1 200	
	Crushed glass (mgm/ml plasma)							
	0	150	0	150	0	150	0	150
Recalcified clotting time (sec )	655	171	205	146	91	87	47	45

0.3 ml of 0.85% sodium chloride solution or diluted thromboplastin was added to 0.3 ml of oxalated plasma. The mixture was then recalcified with 0.3 ml M/40 CaCl<sub>2</sub>. Crushed glass was added just prior to recalcification. The platelet-rich and -deficient plasmas were obtained from the same source.

with crushed glass whether or not the glass was removed by high-speed centrifugation prior to recalcification (Table VII) The recalcified plasma clotting time of platelet-rich plasma was shorter than that of the same plasma rendered platelet-deficient (Table VIII) However, the clot promoting effect of crushed glass was as pronounced on platelet-deficient as on platelet-rich plasma Increasing the surface area of glass was effective in shortening the recalcified plasma clotting time in the presence of diluted rabbit-brain thromboplastin However, as the concentration of this thromboplastin was increased, the clot accelerating action of glass was diminished With potent thromboplastin preparations such as are used in the Quick test, glass had no detectable effect When ovalated plasma was clotted by thrombin,<sup>5</sup> glass had no demonstrable influence on the rate of the reaction

#### DISCUSSION

A number of previous studies suggest that platelets are not essential for the *initiation* of coagulation (2, 3, 4, 5, 6, 7, 8) While some of the data presented are convincing, this conclusion has not been widely accepted It is common belief that platelets are required for the initiation of clotting, but few investigators claim to have produced a spontaneously incoagulable platelet-free plasma from mammalian blood (10, 11, 13)

In the present paper experimental data show that essentially platelet-free plasma was often spontaneously incoagulable in silicone-treated tubes, although relatively prompt clotting invariably occurred in glass tubes The absence of clot formation in silicone-treated tubes indicates that neither effective thromboplastin nor thrombin was produced during the preparation of platelet-deficient plasma However, the occurrence of clotting of centrifuged plasma in glass tubes does not provide absolute proof that the effect of glass surface is not mediated through the platelets, since it is never possible to prove that platelets have been completely removed

If the clot-promoting effect of glass surfaces is brought about by platelet disruption, it seems likely that a high concentration of macerated platelets would be very effective in accelerating coagulation On the other hand increasing the area of the glass surface in contact with severely platelet-deficient plasma would be expected to have relatively

<sup>5</sup> Thrombin, Upjohn Company

little clot accelerating effect. This, however, was not the case. Increasing the glass surface area was much more effective in reducing the clotting time of platelet-deficient plasma than was the addition of high concentrations of platelets which had been macerated on glass surfaces. These observations suggest that the presence of a few remaining platelets is not concerned with the clot-promoting effect of glass surfaces on centrifuged plasma.

While platelets appear not to be essential for the initiation of coagulation, they nevertheless play an important part in the clotting process. When plasma clots on contact with glass, the rate of conversion of prothrombin to thrombin is directly related to the platelet concentration. The clot-accelerating effect of platelets is not appreciable in plasma which is protected from contact with glass and similar surfaces. For rapid conversion of prothrombin both the surface factor and platelets are required.

Plasma which has been stored for relatively short periods of time may not clot on contact with glass. This observation suggests that the clot-promoting effect of glass is mediated by a labile component of plasma. Tests to determine the reactivity of plasma to glass surfaces evidently should be carried out while the plasma is fresh.

Platelet-free plasma is definitely hypo-coagulable as compared to normal plasma. In experiments to determine whether such plasma contains all of the substances necessary for spontaneous coagulation, optimal conditions for clotting must be provided. Those investigators who have reported that platelet-free plasma is spontaneously incoagulable have apparently not tested their plasmas under such optimal conditions. In the reported experiments (10, 11, 13), sub-optimal temperatures and minimal glass surface area have been employed. The experiments described in the present paper show that if coagulation of platelet-deficient plasma is sufficiently delayed, the plasma does in fact become spontaneously incoagulable, presumably because of deterioration of a plasma component.

Students of blood coagulation have for years been troubled by the problem of complete removal of platelets from plasma. A recent report (10) implies that many hours of centrifugation at high gravitational forces are required to remove all of the platelets from normal plasma.

However, in the present experiments approximately 99.995% of the original number of platelets in normal blood were regularly removed by centrifugation for only 10 minutes at about 17,500 g. Furthermore, on direct microscopic observation of the undiluted centrifuged plasma the few platelets remaining were observed to settle to the surface of the counting chamber. No evidence was found to support the belief that these platelets were remarkably less dense than the average platelets. It seems likely that these platelets were derived from the centrifuged sediment as a result of the slight agitation inevitably produced by withdrawal of the supernatant plasma.

The manner in which glass surfaces promote coagulation of platelet-free plasma remains to be clarified. It appears that on contact with glass, plasma undergoes a change in which effective thromboplastin is made available. Studies on patients with certain disorders of blood coagulation (8) make it seem likely that a specific protein is present in normal blood as an inactive thromboplastin and is converted to an active form on contact with a glass surface. No evidence was found that glass surfaces affect the final stage of coagulation.

#### SUMMARY

Normal human plasma of varying platelet concentration was prepared without anticoagulant by centrifugation of blood in silicone-treated apparatus. Essentially platelet-free plasma clotted relatively promptly after transfer to glass tubes, but in silicone-treated tubes coagulation frequently did not occur.

Increasing the glass surface area in contact with plasma shortened the clotting time markedly regardless of the platelet concentration. Crushed glass was more effective in shortening the clotting time of platelet-deficient plasma than was a suspension of macerated platelets.

The clot promoting effect of crushed glass on centrifuged plasma seems better explained by an alteration produced in the platelet-free plasma itself rather than by disruption of a few remaining platelets. After storage for relatively short periods of time, plasmas often failed to clot on addition of crushed glass.

In plasma clotting in contact with glass, the rate of conversion of prothrombin was directly related to the platelet concentration.

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## BOOK REVIEWS

*Conditional Reflexes and Neuron Organization* By JERZY KORNORSKI Cambridge University Press, 1948, pp 267 + XIV, 18 s

The reaction to Pavlov's investigations by the conditional reflex method on the "higher nervous activity" has been a curious and interesting one. For a long time little attention was paid to his discovery at the turn of the century of the conditional reflex, partly on account of the language difficulty, partly because of the lack of scientific intercourse between Russia and the West. When Pavlov's concepts reached this country they were adopted by some psychologists as the nucleus of a new school of thought—Behaviorism, the chief exponent of which was John B. Watson. The overzealousness of its proponents in denying the existence of everything not measurable has caused this school to fall into disrepute. At the present time the opinion in this country of Pavlov's work ranges from an active hostility to its being hailed as one of the greatest discoveries in the history of science. There are many reasons why this work has not been actively taken up in this country since Watson carried out some experimentation by this method on the human being at the Johns Hopkins Hospital. One reason is the lack of trained investigators, another is the general reluctance of physiologists to undertake the chronic experiment requiring a study period of months or even years with all the difficulties of maintaining healthy animals over their life span. In spite of the general acceptance of the importance of the conditional reflex, the lack of active investigators in the field, as well as the novelty of the concepts has resulted in the failure of Medicine to incorporate the data and the concepts into physiology, neurology, psychiatry or even psychology.

Kornorski has written a masterful criticism from a constructive point of view of the Pavlovian concepts. In fact this is one of the few worthwhile evaluations that has been given of the Pavlovian work. Kornorski's purpose is to integrate Pavlov's data with that of Sherrington "in the hope that this work will do something to bridge the gulf between their respective achievements" (from his dedication of the book). His treatise is practically essential for any serious student of Pavlovian Methodology. He goes into detailed discussions of many of the basic Pavlovian concepts, showing their relationships to Sherrington's work on spinal reflexes. With rare insight and with the competence of a first-class investigator in the field he has given us not only a scholarly but probably the most important evaluation we have of the Pavlovian work. Kornorski makes many suggestions as to how the Pavlovian concepts should be re-cast to fit better with Sherrington's material. Especial credit is due Kornorski for having composed this book in the wake of the destruction of his native land (Poland) as well as of his own laboratory.

W. H. G.



*Clinical Orthoptics* BY MARY EVERIST KRAMER, ERNST A W SHEPPARD AND LOUISA WELLS-KRAMER 475 pp \$8 00 *The C V Mosby Co*, St Louis, 1949

This book has been written as a textbook for orthoptic students and as a reference book for graduate orthoptic technicians. It fulfills both of these functions well. The entire problem of orthoptics has been considered both from the clinical standpoint and also from the point of view of the basic sciences. There has been a careful selection of material so that all of the phases of orthoptic work are discussed.

The style of writing is clear and concise, and the book is easy to read. The questions at the end of each chapter should prove a great help to the orthoptic student in systematically reviewing the topics covered. As a whole the illustrations are excellent, but some of the sketches in Chapters 1 and 2 are unnecessarily diagrammatic. Each chapter of the book contains an excellent list of references where the student can obtain more detailed information.

There has been a great need for such a book on orthoptics written expressly for the orthoptic technician.

W C O

*Campbell's Operative Orthopedics* BY J S SPEED AND HUGH SMITH, Second edition, 1643 pp \$30 00 2 Volumes, *C V Mosby Co*, Publishers, 1949

The second edition of Campbell's Operative Orthopedics, like the first edition, is an excellent compendium of operative procedures used in orthopedic surgery. For the student, interne and resident, the value of such a compendium is obvious. For the specialist, it is important that a good publication of this type be available. In a field in which there appear in rapid succession new operations and new techniques, it becomes necessary to have a satisfactory reference book. The present edition constitutes an up-to-date presentation which is particularly valuable because the procedures enumerated are credited to those who described them and the original articles are listed for reference.

There are many improvements in the new edition. The material presented is brought abreast of the current time, with the result that there is much more printed matter. Since this is bound in two volumes, the handling of a single large cumbersome book is avoided.

Many new and excellent illustrations have been added, and on some special subjects, new chapters have been included. Several sections have been written by men who are particularly well qualified and some of these sections are very well done. But the greatest change is probably in the literary form. Instead of the compact, almost laconic, style used in the earlier edition, there is a fluency that makes the text more readable. Furthermore, the authors make the presentation a more personal one by discussing their own experiences with some of the procedures described and the reader profits by their carefully considered, as well as extensive, observations.

I W N

*Fundamentals of Internal Medicine*—3rd Edition BY WALLACE M. YATER 1451 pp \$12.00 Appleton-Century-Crofts, Inc., New York

What is the purpose of a one volume textbook of medicine? In Dr. Yater's view, it should "make readily available in simple form the essentials of internal medicine" in accordance with the concept of "simplification and conciseness with elimination of theoretical and redundant material." To this end, Dr. Yater and his associates have written a well-integrated, readable book, emphasizing clinical descriptions and the essentials of therapy. Many clear tables and illustrations are included, and the physical format of this volume is pleasing to the eye. Little space is spent on the mechanisms of the production of symptoms, nor on the problems of etiology and pathogenesis. These are frequently dismissed with a few short sentences which fail to introduce the student to the many unsolved problems in internal medicine. Moreover, these didactic synopses are frequently misleading. For example, hemophilia is said to be associated with "an increased resistance of blood platelets to disintegration", a view far from universally shared. Nor is the life span of the normal red blood cell thought to be 2 to 6 weeks by most observers. This text is an excellent précis of symptoms and signs, but will not help the student to think in terms of the physiologic mechanisms involved.

O D R

*Medicine of the Year 1949* Editorial Direction, JOHN B. YOUMANS, M.D., Dean, College of Medicine, University of Illinois 143 pp \$5.00 J. B. Lippincott, Phila., Pa.

An immediate reaction to a book which attempts to give the "spot news of medicine", which attempts to furnish the "latest bulletins that indicate the direction in which any phase of medicine is going at the moment", is for many reasons not particularly favorable. Perhaps it is that one recognizes the inherent difficulty of evaluating advances within the year in which they arrive. Yet, after reading through "Medicine of the Year", one is forced to the conclusion that the authors and particularly the editor, have done an excellent job. So diverse is the material that it is, of course, not possible for one person to adequately evaluate the different contributions to internal medicine, obstetrics, pediatrics and surgery with all of its specialized branches. However, within the limits of this reviewer's knowledge, whenever data presented is debatable the authors have so indicated, whenever questions are unsettled there has been no attempt to force them into a mold of decision. At the same time, recent clear-cut advances are presented concisely and factually. The recent evaluation of antibiotics is carefully covered throughout, without any mistakes being apparent even some time after the original articles were apparently written.

There are a few scattered misprints and misspellings, but these are minimal. It will, perhaps, be more difficult to write the issue next year when there will be less reason to go back a few years in evaluating recent progress, but certainly this first try was worthwhile and the next issue will be looked forward to with interest.

F B B

*Nutrition and Diet in Health and Disease* BY JAMES S MCLESTER 5th edition, 800 pp \$9 00 W B Saunders, Phila, Pa

*Nutrition and Diet in Health and Disease* comprehensively reviews the present state of knowledge of this subject Dr McLester discusses the physiology of digestion, the metabolism of food, the composition of common foodstuffs, and the dietetic requirements of normal adults and infants The author then describes the diets suitable for the treatment of disease In each case, a brief summary of knowledge concerning alterations in metabolism is followed by a general discussion outlining suitable dietetic regimens, and the rationale for their use In many instances, sample menus are provided as well The text is supplemented by many tables, the most useful of which give the composition of foodstuffs

This is the fifth edition of this excellent and most readable book, and most of the text has been brought up to date As is almost inevitable, the revision has been more thorough in some sections than in others The use of rice and low salt diets for the treatment of hypertension is discussed and evaluated with commendable caution However, no mention is made of the use of detergents to facilitate intestinal absorption In general, the dietetic recommendations are sound In a few cases, however, one may take issue with the author's point of view For example, a diet rich in calcium is not ordinarily believed to be of value in hemophilia, a disorder in which calcium metabolism is not abnormal

Each chapter is followed by an excellent bibliography, and each section by a list of pertinent reviews covering the subject discussed This book can be highly recommended as a text useful to students, practitioners and dietitians It is unfortunate that many misprints are present, and the type of many of the tables and menus is badly blurred

O D R

*Observations on the Pathology of Hydrocephalus* (Medical Research Council—Special Report Series No 265) BY DOROTHY S RUSSELL His Majesty's Stationery Office, London 1949, Price—Six shillings net, 138 pages

This masterly monograph from the hand of Dr Dorothy Russell will need no introduction to those who know her classical article of fourteen years ago At that time she demonstrated the keen observation and sound reasoning which characterize the present comprehensive report on hydrocephalus She draws on a wealth of morbid anatomical material in exemplifying the categories of her classification The latter is based on the pathological lesions responsible for the obstruction in the cerebrospinal pathway Indeed, the most important conclusion she draws from her detailed studies is that in every case of hydrocephalus some such form of obstruction can be demonstrated if careful search is made

Amongst the types of maldevelopment productive of hydrocephalus, the author stresses the forking atresias and webs of the aqueduct, and the membranes about the fourth ventricle She reiterates her evidence against the traction hypotheses in the causation of the Arnold-Chiari malformation Her examples of the mechanisms of obstruction associated with neoplasms are well chosen

It is in the consideration of the more obscure forms of hydrocephalus, however, that the thoroughness of her survey seems most impressive. It lends great strength to the view that the vast majority of cases rest on an inflammatory basis. This may follow the extravasation of blood or be an insidiously-developing sequel to actual infection. The common sites of such lesions, often found only by careful search, are described. The implications of this discussion of inflammatory causation will be clear to everyone dealing with the child or adult patient. A minor quiver in regard to the validity of the author's distinction between developmental and acquired aqueductal atresia does not detract from this.

In summary, this monograph is strongly recommended to all, and its author warmly congratulated. The quality of the illustrations is in keeping with the subject material.

J W M

*Studies on Hookworm Disease in Szechwan Province, West China* BY K. CHANG AND CO-WORKERS. American Journal of Hygiene Monographic Series no 19, May 1949. Johns Hopkins Press pp 152, \$3 00

There is a prevalent tendency on the part of the educated physician and layman to believe that the battles against some of the common scourges of mankind have been won. The campaigns against hookworm in this country, successful as they were, should not blind us to the fact that in great areas of the world this simple preventable disease remains one of the most important. A clear example of this situation is presented by Dr. Chang and his collaborators who report on the prevalence of hookworm in West China—in the province surrounding the former capital, Chungking. Given a sandy soil, a moderate temperature without extremes, a sufficient moisture and habits of a people which allow for a constant contact between human feces and the bare skin of man—and hookworm becomes a major problem. Such is the case in the corn-sweet potato regions of Szechwan, where the incidence of positive stools approaches 100%. Much more important, as was realized years ago by the Rockefeller Foundation workers, is the parasite load of the community. It is more important to know if a number of people are carrying heavy loads of parasites, than it is to know how many of them are infected. In certain areas of this province, well over 50% of the population carried more than 3000 hookworm eggs per cc. stool. The exact importance of this is of course difficult to assess, for the combination of heavy infection plus a poor diet is the usual combination. However, roughly, 10% had geophagia, and the hookworm load was directly correlated with the percent of hemoglobin, which was often very low.

The variable effects of slight differences in horticultural practices is brought out by a difference in the problem of hookworm infection in Eastern China, where some twenty years ago Dr. Cort and collaborators demonstrated that the cultivation of mulberry trees was associated with a high incidence of hookworm. In contrast in Szechwan, the disease is not particularly serious in mulberry areas. This is because, in the latter, mulberry trees are scattered in different fields, are not inundated with night soil in the same way, and because the trees are allowed to

grow This latter means that the leaves have to be picked by climbing a ladder In Eastern China, the opposite of all of these conditions leads to a severe hookworm problem

To make the report living the authors have given a number of case histories "Li, by name, male, a farmer, aged 26, came to our field clinic on August 19th, 1941, for the treatment of *Lan Hwang Ping* (lazy yellow disease) The patient was very weak, extremely anemic, exceedingly pale, expressionless, the voice was low, he breathed with difficulty but was mentally clear He was exceedingly edematous all over the body, there was extreme edema of the lower extremities, his legs were very puffy and heavy and he could not walk with ease The scrotum and tip of the penis were also extremely edematous Fecal examination showed the following Protozoa, negative, *Ascaris*, negative, *Trichuris*, 300 eggs per cc, hookworm, 45,400 eggs per cc of feces (three counts were made and averaged) A total of 637 worms, of which 602 were *Ancylostoma duodenale* and 35 were *Necator americanus*, were collected from the patient His hemoglobin was only 10%"

This is a good, well-illustrated and worthwhile monograph which should be at least perused by all who are actively interested in the health of the world

F B B

*The New York Academy of Medicine Its First Hundred Years* PHILIP VAN INGEN, M D 573 pp \$10 00 Columbia University Press, Morningside Heights, New York

This is an interesting and amusing chronicle of the meetings and doings of the "fellows" of the New York Academy of Medicine It does not pretend to be history in the broad sense, and one may search in vain for information about some of the famous medical men of New York beyond that which has a direct bearing upon their activities within the Academy

Yet along with the amusing anecdotes and disagreements as to who should be censured for "seeking the advice and consulting with a homeopath", there is real information concerning the opinion of a body of the leading medical men at a particular time The Academy was founded at the turn of the century It was also at this time that Shattuck wrote his famous report on sanitary conditions in Boston and John Snow in England was just beginning to write about the contagiousness of cholera Thus it is of interest to read about the discussions on cholera in the year 1849 "The year might well be called the cholera year" for little else was discussed in the months of February, March, and April

Dr Stewart stated that in connection with the origin and mode of propagation of the disease and particularly with that form of it which prevailed at the Quarantine at Staten Island, at the City of New Orleans, and in the "Western Country," circumstances had occurred which were not yet fully and satisfactorily explained He therefore moved, and it was resolved, "that it is premature and inexpedient

for this Academy to pronounce at the present time any positive opinion in regard to the contagious or non-contagious nature of cholera "

However, in 1866 Dr Harris, an experienced health authority, reported on "Sanitary Police Measures 2 " He insisted that cholera was spread through the diarrhoeal discharges of patients, that disinfection was a simple matter, and rigid regulations should be enforced to disinfect permanently all ejecta, and to provide strictest cleanliness of person and premises, including privies, water closets and cess pools If this were done there should be no fear or hesitancy in caring for the sick At the same time Dr Squibb reported on disinfectants, the best being heat and chlorine for the effluvia, together with lime, and a mixture of lime and charcoal For disinfection of cellars smoke from green wood was best

The Academy was founded in 1846 with several objects in view "First, the separation of the regular from irregular practitioners Second, the association of the profession proper Third, the promotion of the character, interests and honor of the fraternity, etc , and fourth, the cultivation and advancement of the science, by our united exertions for mutual improvement and our contributions to medical literature "

Since this book is more a chronicle of the meetings, it is not always easy from this record alone to see to what extent the Academy has attained each one of those goals Admittedly the importance of the different points has changed At any rate, the book makes for enjoyable reading

F B B

## BOOKS RECEIVED FOR REVIEW

- Advances in Surgery* BY WILLIAM DEW ANDRUS, Chairman, Editorial Board  
554 pp \$11 00 *Interscience Publishers, Inc 215 Fourth Ave New York 3*
- Clinical Cystoscopy* BY LOWRAIN E MCCREA in two volumes, 2nd edition 1152  
pp \$28 00 *F A Davis Co, Philadelphia 3, Pa*
- Diagnostic Tests for Infants and Children* BY H BEHRENDT 529 pp \$7 50 *Inter  
science Publishers, 215 Fourth Ave New York 3*
- Medical Etymology* BY O H PERRY PEPPER 263 pp \$5 50 *W B Saunders Co*
- Oral and Dental Diagnosis* New 3rd edition BY KURT H THOMA 563 pp \$9 50  
*W B Saunders Co W Washington Square, Phila 5, Pa*
- Psychosomatic Medicine* 2nd edition BY EDWARD WEISS AND O SPURGEON ENG  
LISH 803 pp \$9 50 *W B Saunders Co W Washington Square, Phila 5, Pa*
- The Premature Infant Medical and Nursing Care* BY JULIUS H HESS, M D AND  
EVELYN C LUNDEEN, R N 381 pp \$6 00 *J B Lippincott Co, E Washington  
Square, Phila 5, Pa*
- Geriatric Medicine* 2nd edition BY EDWARD J STIEGLITZ 773 pp \$12 00 *W B  
Saunders Co W Washington Sq, Phila 5, Pa*
- Bensley's Practical Anatomy of the Rabbit* 8th edition BY E HORNE CRAIGIE,  
PH D 391 pp \$4 00 *The Blakiston Co, Phila 5, Pa*
- Female Sex Endocrinology* BY CHARLES H BIRNBERG 134 pp \$4 00 *J B Lippin  
cott Co E Washington Sq, Phila Pa*
- Medicine of the Year 1949* Editorial Direction of JOHN B YOUMANS, Dean, College  
of Medicine, University of Illinois 143 pp \$5 00 *J B Lippincott Co E  
Washington Sq, Phila Pa*

# STUDIES OF RESPIRATORY AIR FLOW<sup>1</sup>

## 1 SIGNIFICANCE OF THE NORMAL PNEUMOTACHOGRAM

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The series of investigations to be reported here and in succeeding papers is chiefly concerned with the exchange of gases between the alveolar atmosphere and the ambient air and with the pathological conditions affecting it. The frequency with which this phase of respiratory function is primarily affected by common pathological conditions and the general lack of information on the subject have been the stimulus for undertaking this work.

### RESPIRATORY MECHANICS

The physical characteristics of the flow of air through the respiratory tract may be looked upon as reflections of the physiological apparatus producing and controlling this flow.

Air moves in or out of the lungs because of the existence of a pressure gradient between the lungs and atmosphere. The velocity of this flow is determined by the relationship between this pressure gradient and the resistance of the tract through which the air passes. This pressure gradient is ordinarily produced by the movements of the diaphragm and the thoracic cage (the force of these movements transmitted to the lungs through the pleural fluid). The elasticity of the lungs and thoracic wall and the intra-abdominal pressure add to or detract from the force of these movements. The movement of blood within the thorax or abdomen and the viscosity and inertia of the structures contained therein resist the forces exerted by the diaphragm, the intercostal muscles, and the elastic fibers of all of the involved structures.

<sup>1</sup>Work done with the cooperation of Dr. Ross McLean and the Respiratory Laboratory of the Department of Medicine of the Johns Hopkins Medical School, and the technical assistance of Mary Linderman.

This work has been supported by Grants in Aid from the U. S. Public Health Service.



The resistance to air flow offered by the respiratory tract is a function of the length, shape, and cross sectional area of the conducting tubes (1) (in part determined by the surrounding musculature, the thickness of the lining mucous membrane and the fluid lining the

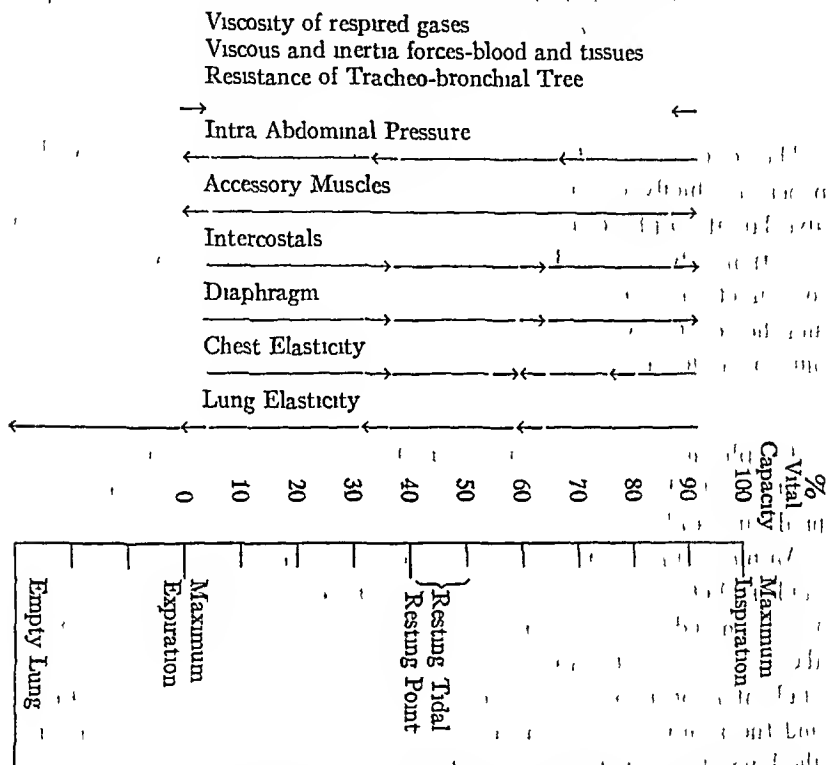


FIGURE 1 Forces involved in the dynamics of respiration. Note change in direction of force of chest elasticity at about 70% of vital capacity, Rahn et al. (19)

tubes) One might think that this resistance would be greater during expiration when the bronchial lumen is smaller, but the decreasing length of the bronchi which occurs simultaneously maintains a constant resistance during the entire respiratory cycle. These changes in the bronchi are easily observed during bronchoscopy and have been studied by other methods (2). The viscosity of the respired gases also plays a part in the resistance to their flow (Fig. 1).

The forces brought into play by the muscles involved, and the

rhythm, at which they function are determined by a multitude of physiological controls including the autonomic nerve endings within the respiratory tract and elsewhere, the pressure in the carotid artery at the level of the carotid sinus, the  $O_2$  and  $CO_2$  tensions in the arterial blood, and other more or less well understood controlling factors

TABLE I  
*Age distribution of subjects studied*

	NORMALS	TOTAL
Birth to 24 hours	3	4
25 hours to 30 days	9	11
31 days to 60 days		2
61 days to 6 months	5	6
7 months to 1 year	1	3
13 months to 2 years	2	2
25 months to 9 years	2	12
10 years to 14 years		7
15 years to 20 years	3	6
21 years to 30 years	19	24
31 years to 40 years	13	22
41 years to 50 years	3	10
51 years to 60 years	1	6
61 years to 70 years		4
Total	61	119

#### THE PNEUMOTACHOGRAM

The pneumotachogram is the record obtained when one continuously measures (usually by a pressure gradient) the velocity of air flow, at the mouth or nose, during respiration, as a function of time.

Since 1925 when Fleisch (3) first constructed a device for the continuous recording of the velocity of flow of respired air as a function of time, numerous investigators have attempted to analyse the pneumotachogram Bretschger (4), Hartwich (5), Hamada (6), Rumpf (7), Gukelberger (8), Silverman (9, 10, 11), Kay (12), Cam (13), and many others have attempted to devise a means of using it to interpret the significance of pathological conditions involving the respiratory tract.

This report and those to follow comprise a study of 119 individuals 61 normal persons and 58 patients with pulmonary disease. The age distribution is shown in Table I.

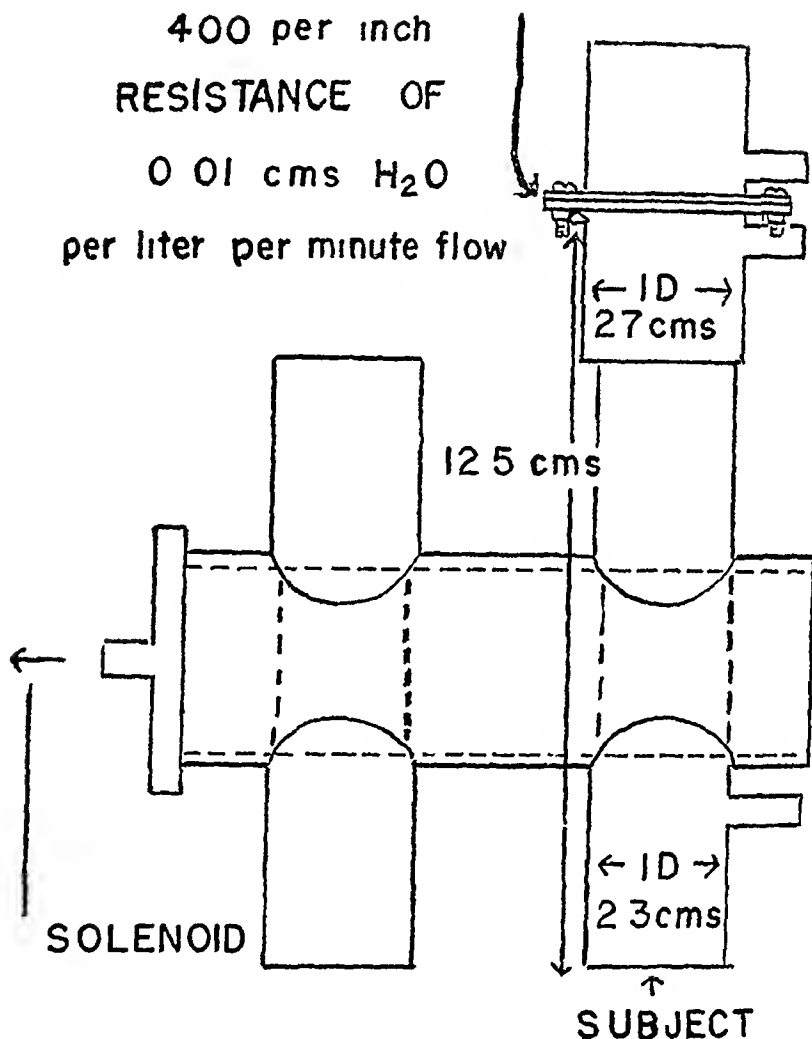
# SINGLE MONEL MESH SCREEN

400 per inch

RESISTANCE OF

0 01 cms  $H_2O$

per liter per minute flow



## DOUBLE FOR USE DURING BRONCHOSPIROMETRY

FIGURE II Diagram of apparatus Pneumotachometers have been calibrated with Schutte Koerting Rotameters Dead space of apparatus is 69 cc Responses to sudden pressure changes equal to those obtained in the studies reported occur in 0 04 seconds

Figure II illustrates the apparatus now in use, the one used in most of the studies herein reported. In certain special experiments and in the earlier work somewhat different pneumotachometers were used

TABLE II

*Nomenclature for analysis of pneumotachogram*

$V_I$	Zero velocity just before inspiration
$V_E$	Zero velocity just before expiration
$V_{DI}$	First point in inspiration when velocity increase in succeeding 0.1 seconds is less than 1/2 velocity increase in the preceding 0.1 seconds
$V_{EI}$	Point at which velocity decrease in succeeding 0.1 second is more than twice that in the preceding 0.1 second
$V_{FI}$	Point of highest velocity during expiration
$T_{IR}$	Time from point $V_I$ to $V_I$ for next cycle
$T_I$	Time from point $V_I$ to $V_I$
$T_E$	Time from point $V_E$ to $V_E$ for next cycle
$T_{DI}$	Time required to reach $V_{DI}$ from $V_I$
$T_{EI}$	Time required to reach $V_{EI}$ from $V_E$
$T_{FI}$	Time required to reach $V_{FI}$ from $V_E$
$V_{RI}$	Peak inspiratory velocity
$V_{RII}$	Peak expiratory velocity (same as $V_{EI}$ )
$V_{MI}$	Peak inspiratory velocity during maximum effort
$V_{MII}$	Peak expiratory velocity during maximum effort
$V_{RII}$	Peak inspiratory velocity — average velocity for inspiration
$V_{RII}$	Peak expiratory velocity — average velocity for expiration
$V_{RII}$	Peak expiratory velocity — average velocity for expiration
$V_{MI}$	Slope of line from $V_I$ to $V_{DI}$ expressed as ccs per (0.1 sec.)
$V_{EI}$	Slope of line from $V_{EI}$ to $V_E$ expressed as ccs per (0.1 sec.)
$V_{FI}$	Slope of line from $V_E$ to $V_{FI}$ expressed as ccs per (0.1 sec.)
$V_{FI}$	Slope of line from $V_{FI}$ to $V_I$ expressed as ccs per (0.1 sec.)
$T_R$	Total cycle time — inspiratory time
$T_I$	
$V_{MI}$	Inspiratory maximum effort peak velocity — resting inspiratory peak velocity
$V_{MI}$	
$V_{MII}$	Expiratory maximum effort peak velocity — resting expiratory peak velocity
$V_{MII}$	

The pressure drops have varied from the highest of 0.03 to the lowest of 0.007 cms H<sub>2</sub>O per 1 liter per minute flow

In order to discuss the pneumotachogram it is necessary to use an intelligible nomenclature. The one used in this paper with appropriate definitions is to be found in Table II.

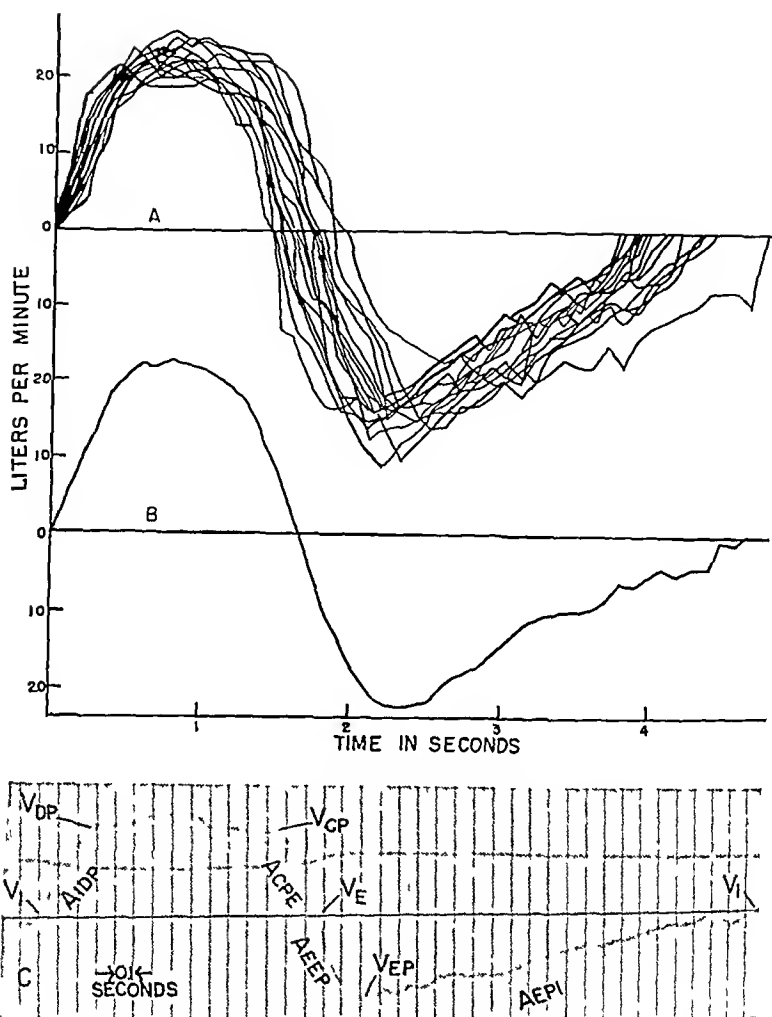


FIGURE III-A Sixteen consecutive cycles, superimposed, on one normal subject  
 B Mean of the above sixteen cycles  
 C Record of one cycle Time lines 0.01 seconds (In all other records time lines represent 0.1 second) Inspiration above zero line

Comparison of consecutive cycles or cycles taken on different days from records on any single subject impresses one with the consistency with which an individual pattern is reproduced (Fig III)

That different individuals have widely differing patterns is easily

seen when one tries to superimpose any subject's pattern on that of another subject (Fig IV)

If one attempts to imitate the pattern of another subject he readily sees that these patterns are either the result of deeply seated habit or of relatively stable characteristics of the individual respiratory system

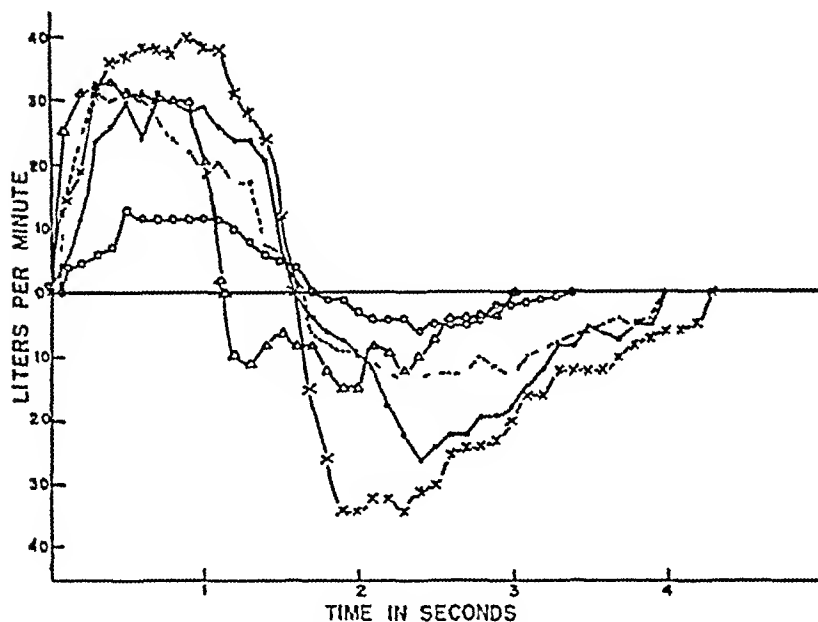


FIGURE IV Superimposition of cycles from five normal subjects

#### ANALYSIS OF THE PNEUMOTACHOGRAM

Certain conclusions, founded on a priori reasoning and borne out by experimental observations, may be drawn from consideration of the subject whose mean pneumotachogram is shown in figure III

##### *1 Action of the inspiratory musculature*

The initial rise to peak velocity  $A_{IDF}$  represents the movement of the diaphragm supplemented by beginning movement of the chest wall. Records taken while mechanically limiting the motion of the abdomen show a partial elimination of the point  $V_{DF}$  and a change in the initial rising line to approach one such as  $A_{ICP}$  (Fig V)

The point  $V_{CP}$  represents the peak action of the intercostals supplemented by continuing but slowing motion of the diaphragm. When the movement of the chest wall is mechanically limited the pattern approaches that one might see in  $A_{IDF}$  and  $A_{DFE}$  (Figure V)

## 2 Transition from inspiration to expiration

Inspiratory effort is terminated rather suddenly as seen by the slope  $A_{CPE}$ . In studying the pneumotachogram it is important to realize

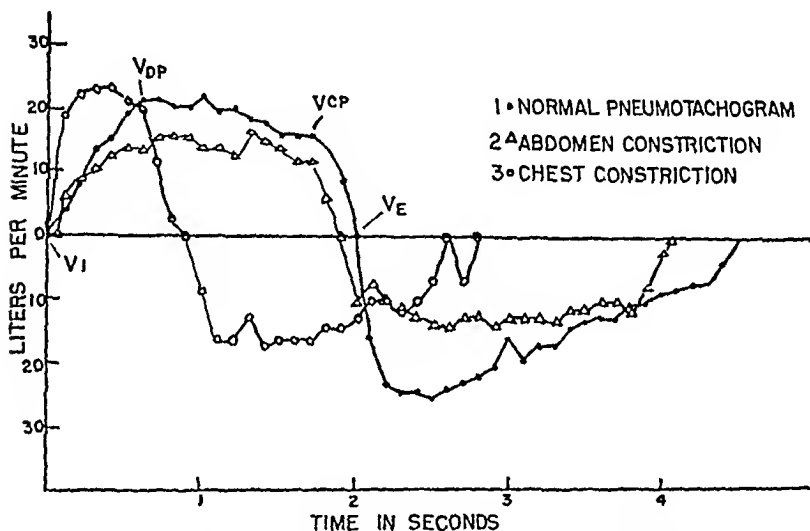


FIGURE V Effects of mechanical limitation of abdomen and chest

that at the point  $V_E$  air has just ceased to move into the lungs and is just beginning to move out. The action of the inspiratory muscles probably begins to be inhibited shortly after the point  $V_{CP}$  but it is likely that all inspiratory effort is not stopped until shortly after  $V_E$ . The reversal in direction of flow occurs when the elastic forces exceed the subsiding force of inspiratory effort.

Under ordinary conditions the sharp slope of the line crossing zero at  $V_E$  is indicative of the fact that the termination of inspiratory effort is a relatively rapid procedure. This phenomenon may be compared to the archer drawing a bow string. He draws the bow string back, at first rapidly, then more slowly. When it is all the way back,

with excellent muscular coordination the archer pauses for a moment to take aim. It is exactly this pause which is ordinarily lacking in respiration. The bow is drawn and released automatically at a given point of tension by a neat trigger mechanism sometimes spoken of as the Hering-Breuer reflex.

### 3 Action of lung elasticity in expiration

Since the continuing force of inspiratory effort beyond the point  $V_E$  is probably negligible and since expiration involves no muscular activity the slope  $A_{EEP}$  results from the simple opposition of the following forces

Resistance to airflow and resistance to thoracic and abdominal tissue distortion

—————

Elastic tension at given degree of inflation.

The peak velocity  $V_{EP}$  is a measure of lung elasticity when considered in conjunction with

- a The pressure gradient associated with such a flow velocity in the individual
- b The volume of inflation of the lungs beyond the resting point of the chest
- c The resistance offered by other viscous and inertia forces

The slope  $A_{EPI}$  is a measure of the decrease in flow velocity concomitant with the change in lung elasticity with decreasing volume. The fact that fall in velocity along this line is almost linear in respect to time indicates that expiration is terminated at the resting volume of the chest (the point at which lung elasticity and chest elasticity are balanced).

A demonstration of the effect of elasticity on the pneumotachogram is accomplished by two simple experiments. When a subject increases his inspiratory volume there is a corresponding increase in the height of the point  $V_{EP}$  and a sharper slope to  $A_{EEP}$  in the ensuing expiration (Fig. VI-A). When the subject stops at the end of a normal expiration and breathes out an additional tidal volume, then relaxes for inspiration (allowing chest elasticity to restore the thorax to its resting position) one readily sees the inspiratory pattern assuming the shape of the normal expiratory pattern (Fig. VI-B).



Twenty of the normal adult subjects (52%) had patterns similar to that shown in Figure III. In nine (24%) the patterns were quite

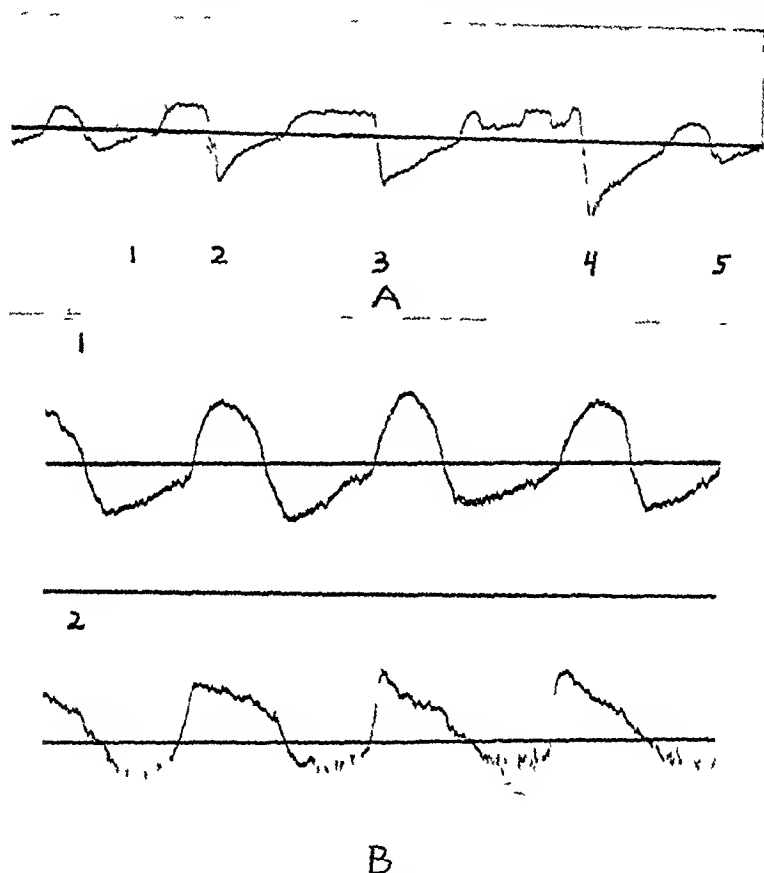


FIGURE VI-A Increasing inspiratory volumes beginning at 1, 2, 3, & 4 expiratory peaks following larger inspirations, & return to normal

FIGURE VI-B Breathing below resting point of chest 2, compared to normal pneumotachogram on same subject 1

similar except for the termination of expiration. In these subjects the slope  $A_{EPI}$  is terminated by an almost vertical line going from flows of 5-20 liters per minute to zero in 0.1 seconds (Fig VII-A). This probably signifies either a failure to obtain a true resting record, or an habitual breathing at a volume slightly above the normal resting

point of the chest. The remaining nine subjects (24%) show a very different pattern in which there are no sudden changes in velocity (note the slow cross-over at  $V_E$ ) peaks are relatively low and expira-

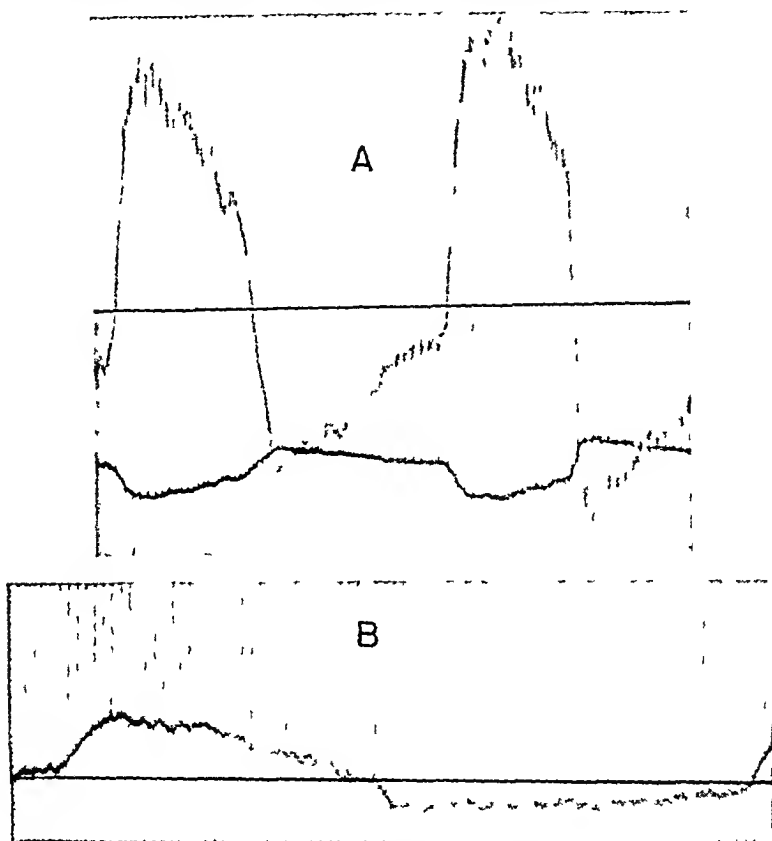


FIGURE VII-A Second type of normal pneumotachogram  
B Third type of normal pneumotachogram

tion and inspiration more closely resemble one another (Fig VII-B). The significance of this type of pattern has not been determined.

No normals in this series have consistently had post-inspiratory pauses (periods of zero flow of appreciable length), but several have had post-expiratory pauses. These appear as a perfectly flat line on zero for 0.1 to 0.4 seconds at the end of expiration, or a zero line punctuated by a brief expiratory or inspiratory puff of low velocity.

Such a pause occurring in one of our normal subjects showed a change in character at a later date (during the seventh month of pregnancy) (Fig VIII)

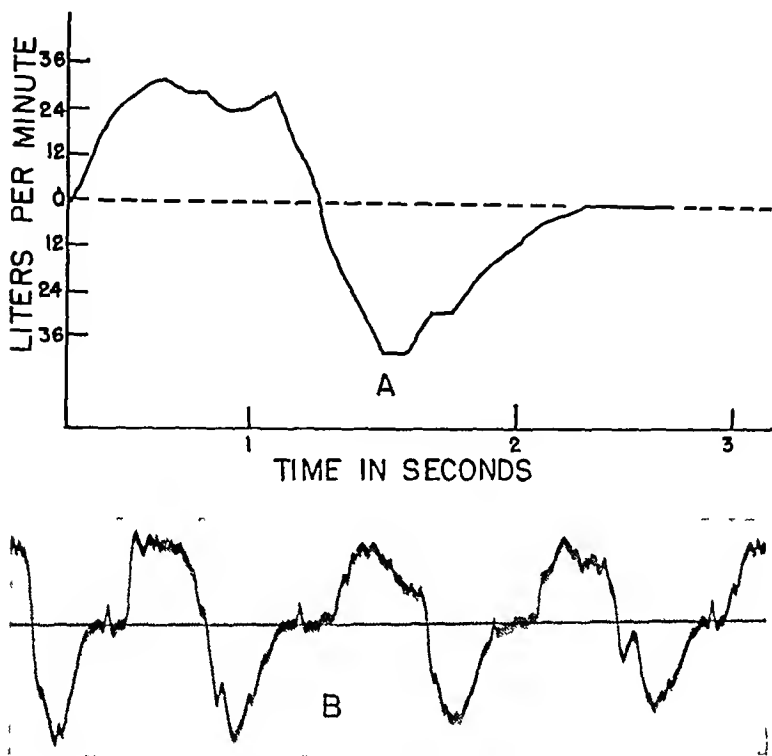


FIGURE VIII-A Before pregnancy  
B During seventh month of pregnancy

Hyperventilation will produce a post expiratory pause in a normal subject not ordinarily having one. It is conceivable that when it occurs regularly the subject is one who, in one sense of the word, chronically hyperventilates.

In figure IX-A is shown a record taken on a normal 24 hour old infant. The peak flows are between 2 and 3 liters per minute, tidal volume is a little over 10 ccs, respiratory rate is a little over 40. The infant patterns, (much more widely varied in shape than the adult), at times are quite repetitive. Figure IX-B shows a record taken at the beginning

of a crying spell in a 16 day old premature infant (1800 grams at birth) Other studies of infants and children will be reported in a later paper

The effects of position sleep, exercise, etc have not as yet been adequately explored Pneumotachograms taken under such circumstances on one normal subject are illustrated in Figure X

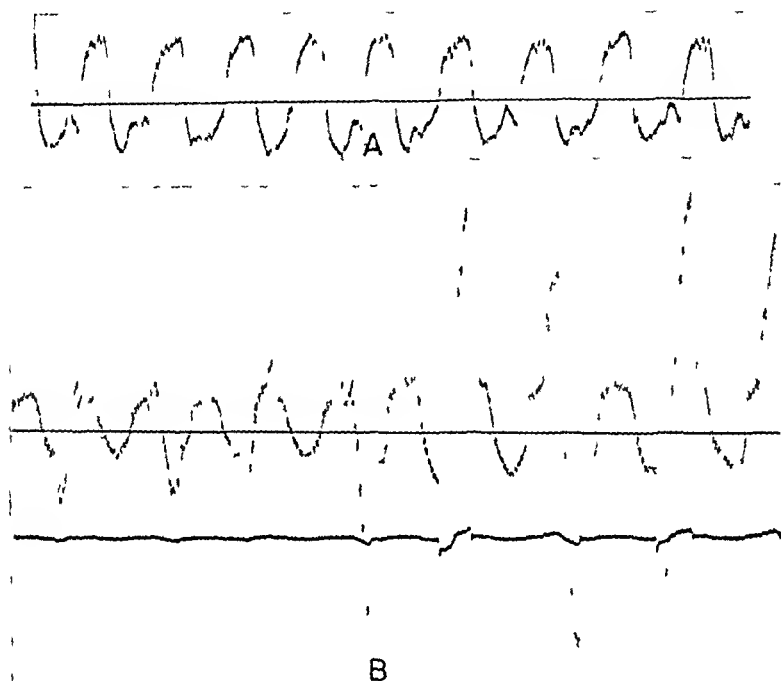


FIGURE IX-A Normal 24 hour infant

B Crying spell in 16 day old premature infant

#### QUANTITATIVE ANALYSIS

An attempt has been made to submit the pneumotachograms studied to various forms of quantitative analysis Bretschger's (4) division of all patterns into three groups according to their approach to the shape of a dome, plateau, or triangle seems to be unsatisfactory The majority of the patterns in this study fit only very roughly into such categories Gukelberger's (8) suggestion that the rates of acceleration

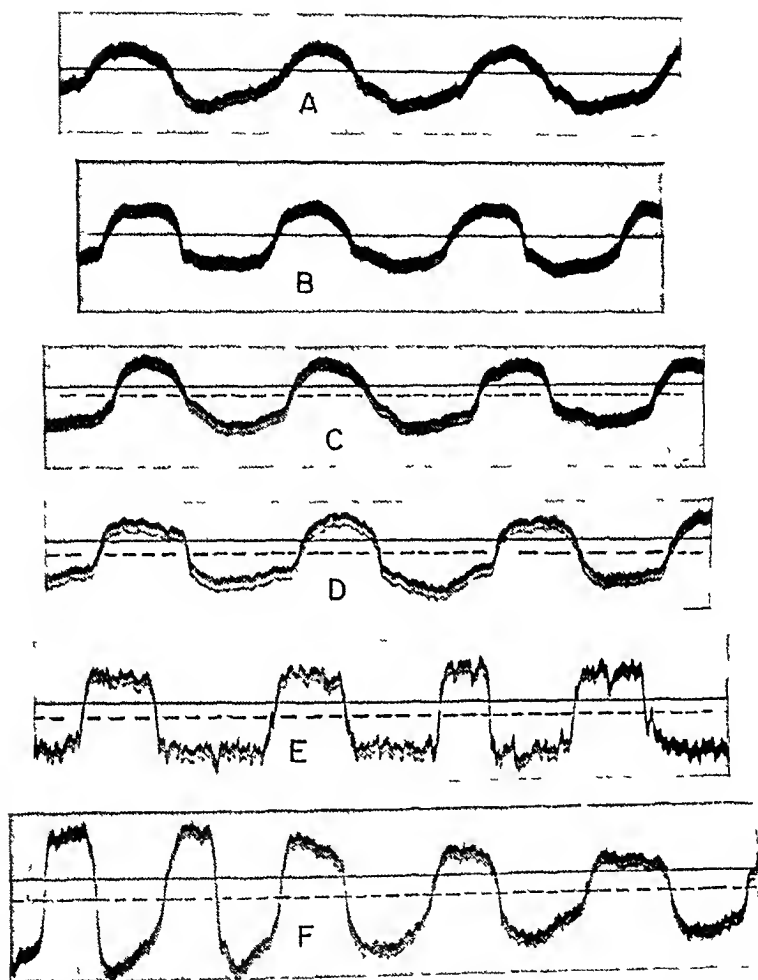


FIGURE X Pneumotachograms during a sitting, b standing, c lying supine, d lying prone, e during moderate exercise of legs, f after severe exercise

Zero passes along top of record in a, in all others it passes through center of record line. In c, d, e & f zero line is dotted

and deceleration be studied is useful but does not consider other significant characteristics. The same objections hold for the methods of analysis used by Silverman and others.

Studies of the subjects herein reported include measurements of

acceleration, velocity and time relationships at various points during the cycle

Patterns which seem quite similar in shape, when submitted to such methods of quantitative analysis yield widely differing figures. Patterns in patients with pulmonary disease (to be reported in a forth-

TABLE III  
*Quantitative analysis of pneumolachograms*  
(For definitions see Table II)

	AVERAGE NORMAL	NORMAL RANGE	RANGE IN PULMONARY DISEASE (24 PTS)	NUMBER WITH PUL- MONARY DISEASE OUTSIDE OF NORMAL RANGE
$T_R/T_I$	2 22	1 57- 2 90	2 07- 3 33	2
$\Delta EEP^*$	12 00	1 43-45 30	2 30-48 00	1
$\Delta IDP^*$	20 00	4 00-72 00	2 50-97 00	5
$V_{RI} \uparrow$	35 00	13 00-78 00	—	—
$V_{RE} \uparrow$	29 00	6 00-67 00	—	—
$V_{RI}$	1 29	0 73- 2 33	0 73-2 27	0
$V_{RE}$				
$V_{RI}$	1 55	1 27- 1 86	1 18- 2 85	8
$V_{RI}$				
$V_{RE}$	1 70	1 15- 2 88	1 17- 2 50	0
$V_{RE}$				
$V_{MI}$	5 00	2 50- 7 55	1 25- 6 15	7
$V_{RI}$				
$V_{ME}$	6 33	1 40-13 30	1 18- 4 25	2
$V_{RE}$				

\* ccs per (0.1 second)<sup>2</sup>

† Liters per minute

coming paper), with obvious variations from the normal configuration, break down into figures which often lie in the range of normal and, even in extreme instances, are not significantly different from those obtained from the normals. As examples some of the figures obtained are listed in Table III.

Possible explanations for the failure of such an analysis to yield significant similarities or differences may lie in the following hypotheses

1 The records studied may not represent true resting pneumotachograms

2 The methods of quantitative analysis may not include the fundamental characteristics of the pattern. Perhaps an analysis of the total shape of the curve is required.

3 Some supposedly normal subjects may not have completely normal respiratory systems or respiratory habits

4 The pneumotachogram may not be a consistently reliable method for study

5 As in other studies of respiratory function the most useful interpretations may be made by correlating the findings in a variety of functional tests. A comparison of function when the subject is at rest with the subject during maximum effort, may be especially useful

#### MAXIMUM EFFORT PNEUMOTACHOGRAM

In an attempt to explore the last of these hypotheses several methods of analysing maximum effort have been tried. Heretofore such studies have been largely confined to the performance of the standard "maximum breathing capacity" test: the attempt to produce, against low resistance, maximum ventilation of the lungs over a period of twenty seconds expressed in terms of liters per minute.

When one attempts to concentrate maximum effort on one expiration or inspiration, peak flows of over 250 liters per minute can be reached in 0.1 second on expiration and a little below 200 liters per minute in 0.23 seconds on inspiration. The slope of the expiratory line is almost perfectly straight whereas the inspiratory slope curves sharply at the top (Fig. XI).

If no other limiting factors were involved, such a subject should be able to perform a maximum breathing capacity with a cycle of 0.33 seconds regardless of the tidal volume resulting. Actually in this subject when the cycle length was 0.33 seconds during such a test the tidal fell below 200 ccs and the ventilation was well below half the maximum breathing capacity for the individual.

When tidal volumes, during maximum breathing capacity tests are charted against the length of the cycle a straight line relationship is found, but this line does not pass through zero. Instead it crosses the time line at about 0.13 seconds (Figure XII-A). Approximately

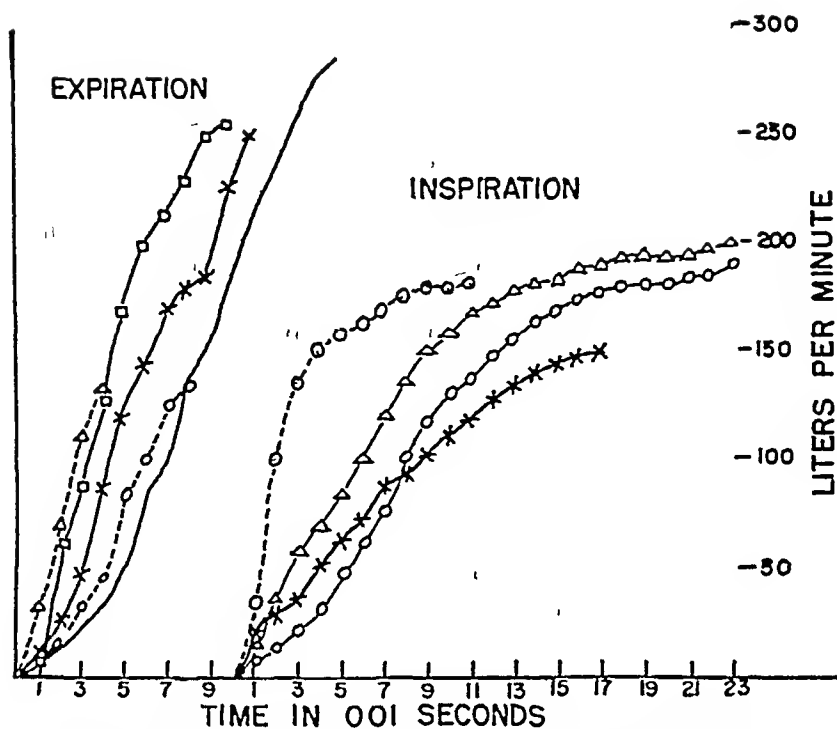


FIGURE XI Chart of single maximum efforts, expiratory and inspiratory

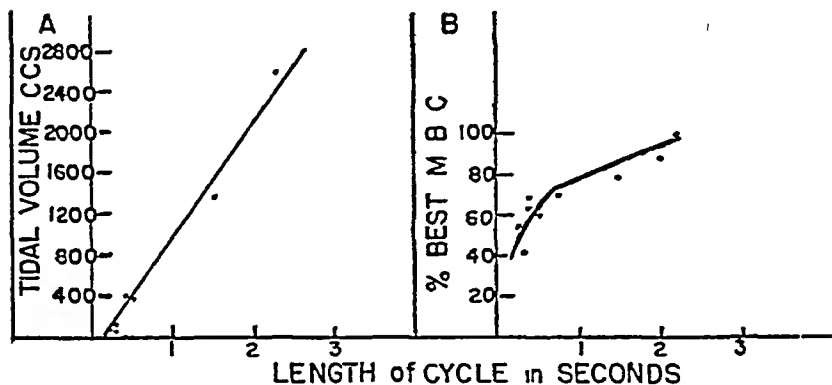


FIGURE XII-A A chart of tidal volume against cycle length during performance of maximum breathing capacity tests

B Maximum breathing capacity charted against cycle length.



this amount of time seems to be required for the neuromuscular apparatus to stop motion in one direction and start motion in the other (14)

If maximum breathing capacity is charted against length of cycle one finds a sharp rise in ventilation with increasing length of cycle until about 0.6 seconds is reached (Figure XII-B). At about this point the line breaks and increase of ventilation is much less with each increase in cycle length. The highest maximum breathing capacity in this normal subject was obtained with a cycle of 2.2 seconds. Ordinarily peak flows can only be maintained for about 0.7 seconds in inspiration and 1.0 seconds in expiration.

These figures may be restated as follows

Length of cycle for maximum breathing capacity in this normal should equal

0.1 seconds time to reach peak expiratory flow

0.23 seconds time to reach peak inspiratory flow

0.13 seconds time to change direction at the end of inspiration

0.13 seconds time to change direction at the end of expiration

---

0.59 seconds

But one must also consider the facts that, on the one hand, the mere change in direction of flow means waste time, and, on the other hand, peak flows can only be maintained for certain intervals. Therefore we add to

0.59 seconds

1.0 seconds maintenance expiratory peak

0.7 seconds maintenance inspiratory peak

---

2.29 seconds

It will be readily seen that these theoretical considerations have led to the first value of 0.59 seconds which coincides with the break in the curve in figure XII-B and 2.29 seconds which is very near the cycle length actually employed by this subject during his most successful effort.

Many poorly controlled factors enter into the ordinary test for maximum breathing capacity: the resistance of the apparatus, the subject's willingness to cooperate, his cleverness of performance, the tendency to choke and cough, etc. It seems reasonable to suggest that a

better understanding of the information sought could be obtained by the study of the maximum effort pneumotachogram and alveolar pressures

The ability of the subject to reach peak velocities, the pressure gradients associated with such velocities, and other physical factors controlling the subject's ability to produce high rates of ventilation should all be studied for a more complete understanding of his ventilatory reserve

In the normal subject maximum effort pneumotachograms consistently reveal rapid rates of acceleration and deceleration at the beginning and end of inspiration and expiration a triangular pattern when the emphasis is placed on rapidity, and a nearly rectangular one when the emphasis is on depth (Figure XIII)

Peaks reached during maximum effort tend to be much higher than those reached in quiet breathing. On the average among the normal subjects studied maximum inspiratory peaks were slightly over five times as great as resting inspiratory peaks whereas maximum expiratory peaks were well over six times higher than resting expiratory peaks

On the average the ratio between resting inspiratory peaks and expiratory peaks was  $V_{RIP}/V_{REF} = 1.29$

#### ADDED RESISTANCE TO BREATHING

Measurement of alveolar-atmosphere pressure gradients is of great importance in evaluating the role of resistance to the flow of air in respiration. Such measurements have been made on all of the subjects studied in the manner reported by Otis and Proctor (15). The method is based on that first used by Vuilleumier (16) the principle of which (along with a sample record) is shown in figure XIV

The simplest example of added resistance to breathing is the change from mouth to nasal breathing. In the normal subject such a change can add sufficient resistance to produce alveolar pressure gradients as much as five times greater than those in mouth breathing

Studies of the effect of added resistance to breathing have been so far confined to one normal subject. The lowest resistance used, 0.1 cms H<sub>2</sub>O per liter per minute flow, is comparable to the resistance of nasal breathing. Other added resistances produced pressure drops

of 0.15, 0.36, and 0.5 cms  $H_2O$  per liter per minute flow. These were all compared to the resistance of the pneumotachometer of 0.01 cms

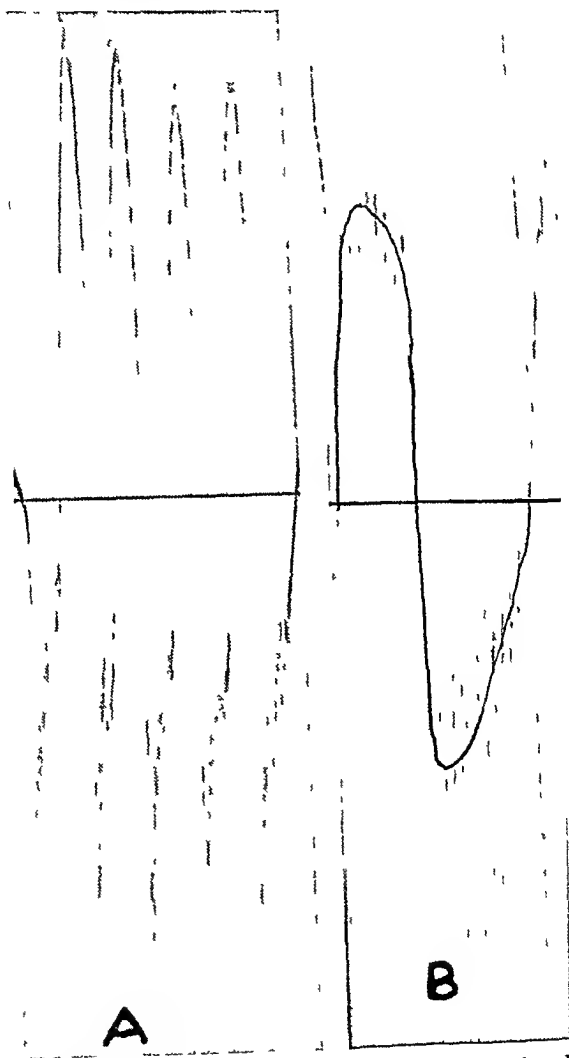


FIGURE XIII Maximum effort pneumotachogram (a) rapid and (b) deep

$H_2O$  per liter per minute flow (Figure XV-A) Records were taken when the subject felt that respiration was quiet (usually about 30 seconds

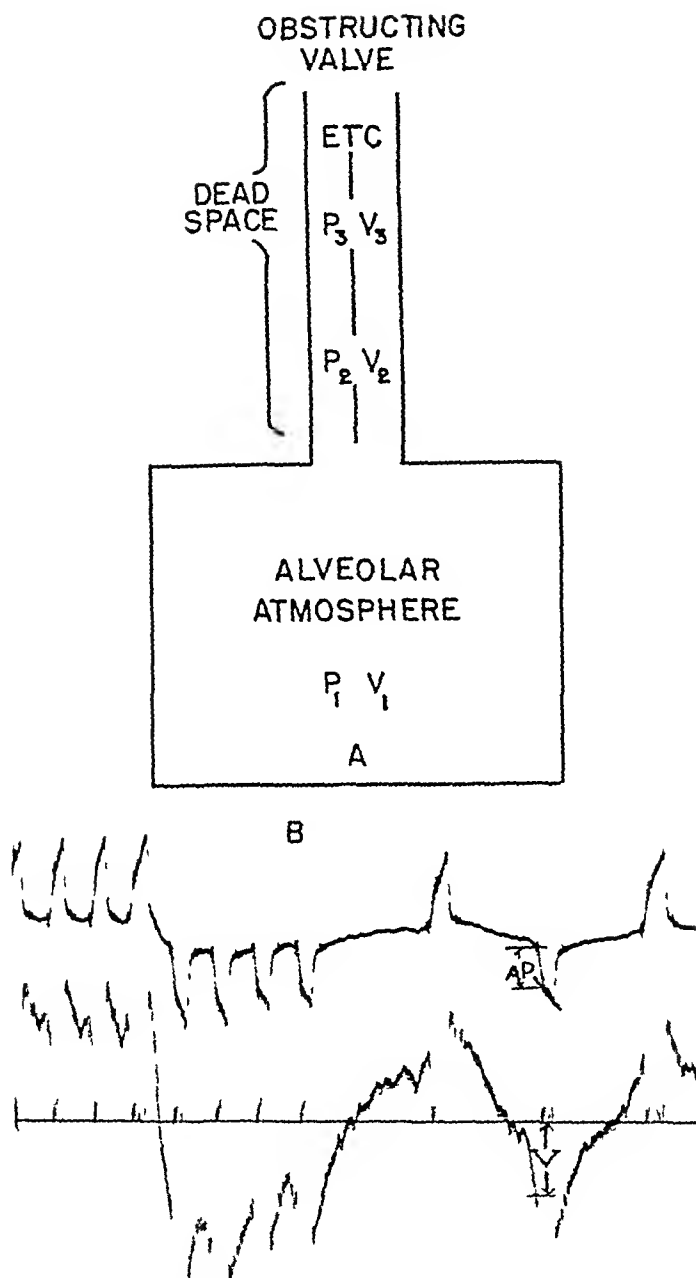


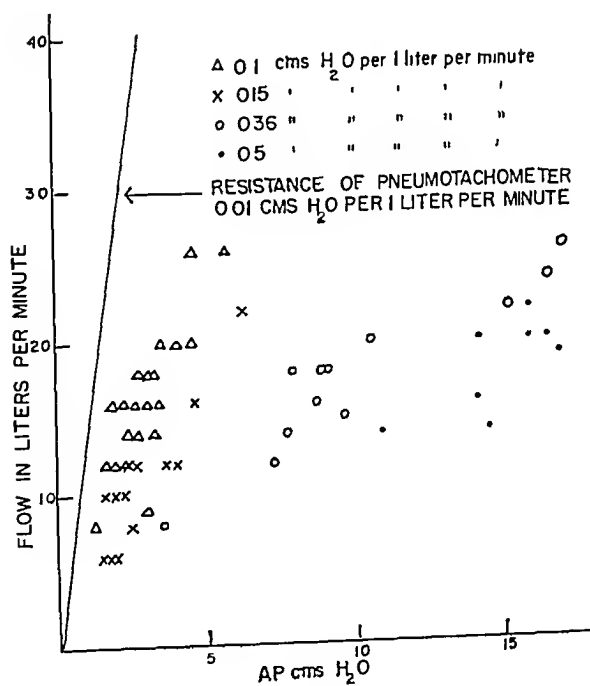
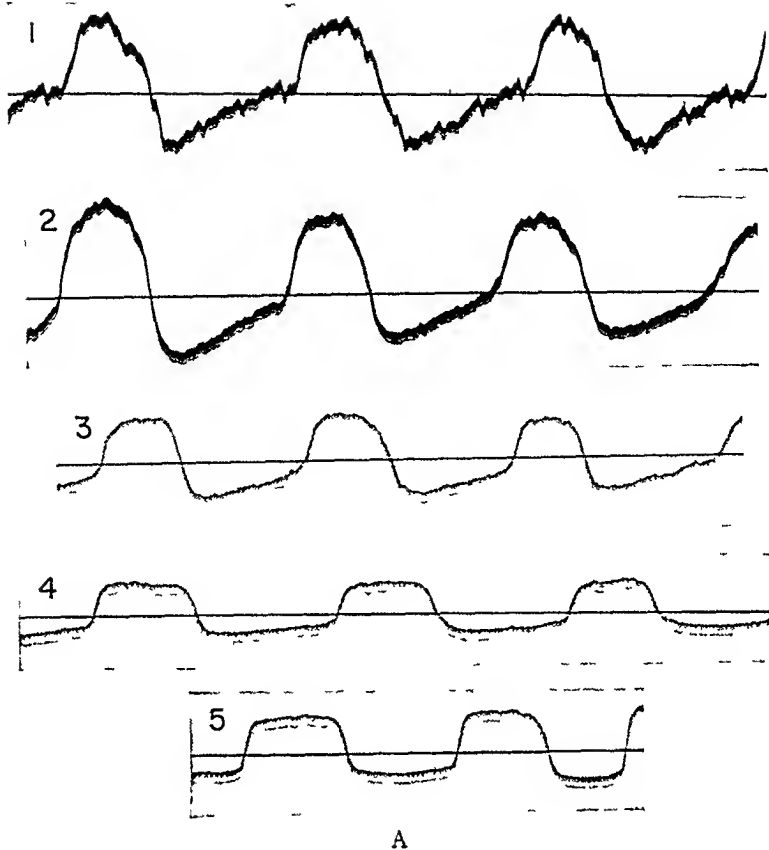
FIGURE XIV Principle of measurement of the alveolar pressure gradient by method of Vuilleumier and record with pneumotachogram

A Sudden obstruction to air flow at the mouth results in rapid equalization of pressure throughout the respiratory tract

The large alveolar volume in comparison to dead space volume causes this equalized pressure to closely approximate alveolar pressure at moment of interruption

$PV$  (equalized pressure and total respiratory volume) =  $P_1V_1 + P_2V_2 + P_3V_3 + \dots$

B Alveolar pressure record (above) and pneumotachogram (below)



B  
FIGURE XV

after starting to breathe through the apparatus) The highest resistance was sufficient to cause definite discomfort to the subject who was seated and at rest

It will be noticed that the lowest resistance added alters only slightly the normal respiratory pattern whereas the additional resistances produce patterns with an almost rectangular wave, expiration being very similar in appearance to inspiration In conjunction with this the subject noted a necessity for employing the respiratory muscles in expiration as well as in inspiration and, a conscious desire to end each phase of each cycle and begin the next

With the highest resistance tidal volume is higher by 14%, respiratory rate more rapid by 40% and the proportion of the cycle spent on expiration is less in comparison to the no resistance pattern by 10% (Tidal volumes without the resistance were about 500 ccs ) The lesser degrees of resistance showed tids smaller, respiratory rates only slightly higher and proportion of time spent on expiration slightly higher than normal

The alveolar pressure gradients accompanying these resistances are shown in Figure XV-B It would seem that, when resistances are such as to increase the alveolar pressure gradient by a factor of five or less, relatively little change in respiratory activity occurs An increase by a factor of ten or more produces changes involving increase in minute ventilation and almost completely active expiration

Of greater practical importance is the radical curtailment in the ability of the individual to produce increases in ventilation with maximum effort (Figure XVI)

Many aspects of this subject have been explored by Silverman (17, 18) and more recently by Cain (13)

In 1946 Rahn et al (19) studied the pressure volume diagram of the chest This work has been repeated using the pneumotachogram for

---

FIGURE XV-A Pneumotachograms on normal subject

- 1 Added resistance of pneumotachometer alone (0.01 cms H<sub>2</sub>O per 1 Lit/Min flow)
- 2 Added resistance of 0.1 cms H<sub>2</sub>O per 1 Lit/Min flow
- 3 Added resistance of 0.15 cms H<sub>2</sub>O per 1 Lit/Min flow
- 4 Added resistance of 0.36 cms H<sub>2</sub>O per 1 Lit/Min flow
- 5 Added resistance of 0.5 cms H<sub>2</sub>O per 1 Lit/Min flow
- B Corresponding alveolar pressure gradients

volume measurements and peak flows after release of obstruction, and the alveolar pressure interrupter for relaxation pressures. The subject would breath in or out any given quantity of air and then be obstructed. He would relax against the obstruction which would then be suddenly

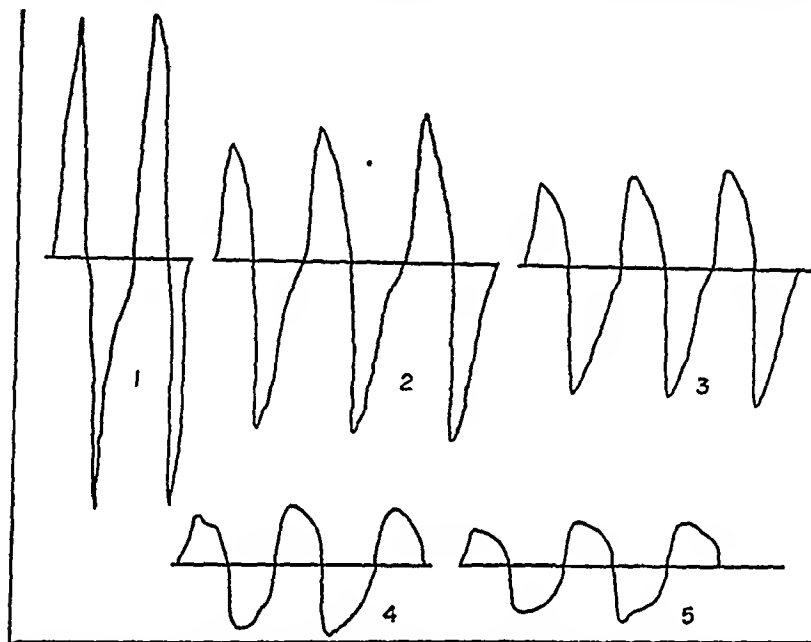


FIGURE XVI Maximum effort pneumotachograms under circumstances of resistance listed in Figure XV-A, 1,2,3,4,5

removed. In Figure XVII are shown the figures obtained and charted across them is the curve obtained by Rahn et al

The pneumotachogram can also be employed in conjunction with a variety of other studies as an aid in the orientation of separate measurements in respect to time (Figure XVIII-A & B). Charting the velocity of flow of respired air against such measurements as intrapleural pressure, volume displacement, acceleration, lung elasticity etc aids in the proper interpretation of these values (20, 21)

#### SUMMARY

1 Experimental analysis of normal pneumotachograms has substantiated the hypothesis that portions of the respiratory pattern are

true reflections of the activity of the respiratory musculature, the effectiveness of lung elasticity, and the manner in which reversal of respiratory effort is accomplished

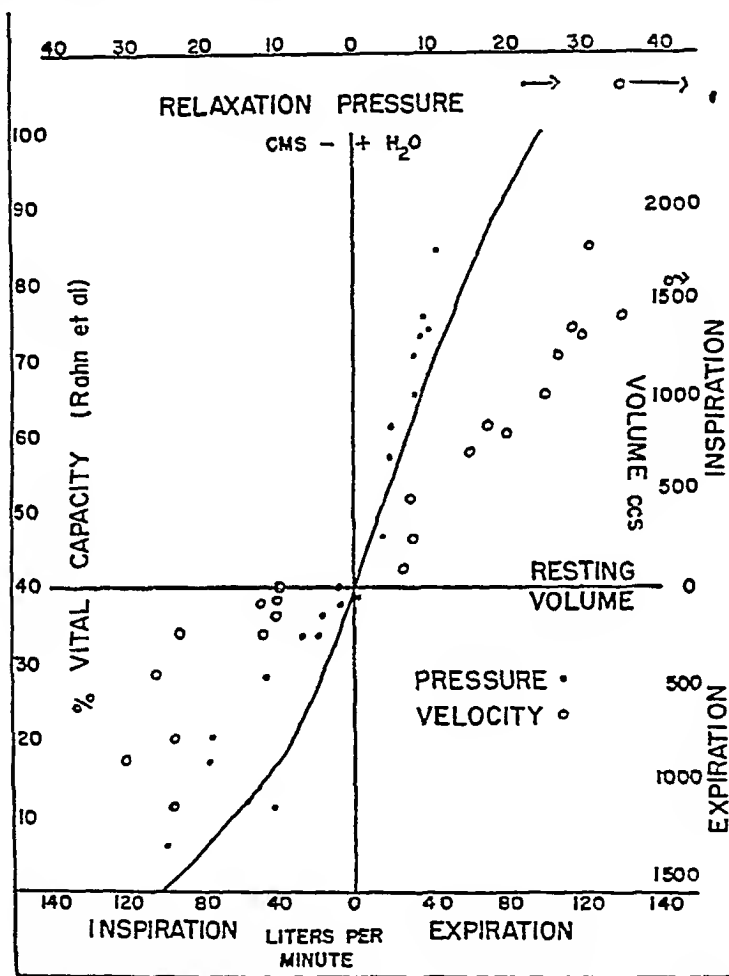


FIGURE XVII Pressure volume diagram of the chest The solid line is taken from Rahn et al (17)

2 Study of records taken during maximum respiratory effort provides information facilitating a classification of the factors controlling the ventilation reserve the ability rapidly to reach high flow velocities,



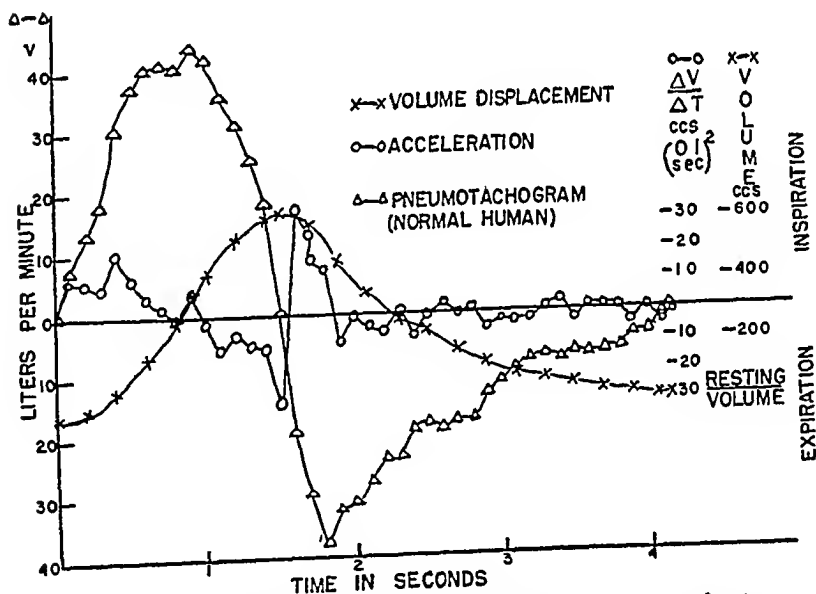
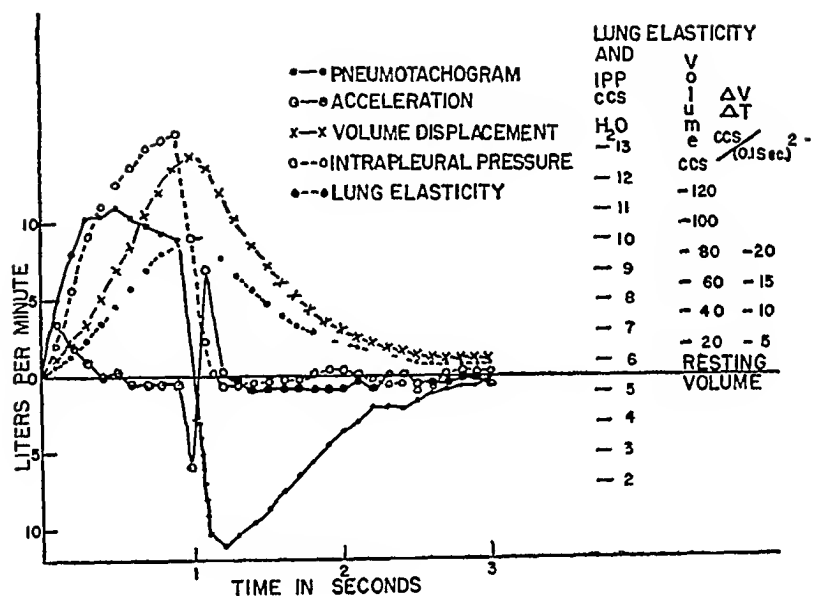


FIGURE XVIII-A Intrapleural pressure, pneumotachogram, acceleration, volume displacement, elastic tension (calculated) Measurements taken on dog under nembutal anaesthesia

B Pneumotachogram, acceleration, and volume displacement on human subject

the time during which such high flow velocities can be maintained, and the time required for reversal of direction of flow of air

3 The pneumotachogram yields information about the manner in which the organism adapts the respiratory apparatus to added resistance to breathing, especially when combined with studies of the alveolar-atmosphere pressure gradient

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# HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE

## I THE NORMAL PATTERN<sup>1 2</sup>

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The histochemical observation that cartilage cells contain glycogen was made almost a hundred years ago. In the intervening years there have been numerous isolated studies applying chemical reactions to demonstrate other constituents in sections of cartilage and bone. No systematic or integrated investigations have been reported, however, nor have adequate supervital techniques been applied. Inasmuch as knowledge of the biochemical reactions as well as the chemical structure of normal cartilage and bone is so meager, we have thought that it would be desirable to apply certain qualitative techniques to these tissues in order to obtain an overall picture of the normal pattern which could then be used as a base line in an investigation of certain abnormal states produced by one means or another, with particular stress on vitamins and hormones. The present paper serves as a review and, in addition, will present certain unreported observations which have been carried out on the cartilage and bone of normal rats and guinea pigs and humans at autopsy.

No details of the methods used will be presented here. A description of or reference to each will be found in its appropriate place.

### ENZYMES

*Cytochrome Oxidase* In 1885 Ehrlich (11) initiated experimental intracellular chemistry when he noted the development of a blue color in many tissues of animals which had been injected with alpha naphthol and dimethylparaphenyldiamine, now called the nadi reagent. An enzyme, first named indophenol oxidase, was shown to be responsible.

<sup>1</sup> A part of the observations herein presented were reported at the annual meeting of the Association of American Pathologists and Bacteriologists, March, 1948 (14).

<sup>2</sup> Aided by a grant from Mead Johnson and Company.

in the presence of oxygen, for the formation of the indophenol blue dye as a result of the oxidation of dimethylparaphenyldiamine and its reaction with alpha naphthol. More recently, evidence has accumulated that indophenol oxidase is cytochrome oxidase. The oxidation of the nadi reagent with the production of indophenol blue may therefore be used as a qualitative test for the presence of cytochrome oxidase in tissues.

In studying this reaction we have used thin, free hand slices of cartilage and bone, usually the costochondral junction or upper epiphysis of the tibia of rats and guinea pigs. Frozen sections, though of course thinner, do not give as active preparations. The tissue is placed in a drop of Krebs-Ringer phosphate solution (pH 7.4) on a slide and to this are added one or two drops of a freshly prepared solution of equal parts 0.1 M alpha naphthol and 0.1 M dimethylparaphenyldiamine (paraaminodimethylalanine) in Krebs-Ringer phosphate solution (pH 7.4). The tissue without a cover glass is then observed under low power of the microscope. Incubation hastens the reaction but is not obligatory. Certain adjunct procedures are necessary as controls. Since cyanide inhibits cytochrome oxidase activity, a drop of KCN (0.1 or 0.01 M in Krebs-Ringer phosphate) is added just before the tissue is brought into contact with the nadi reagent. In addition, as Flexner (13) has suggested, ferricyanide should be added to the washed slice after the maximal development of blue color in order to determine whether the reagent has actually entered the tissue. Ferricyanide, of course, oxidizes the nadi reagent to indophenol blue.

When the nadi reagent comes in contact with slices of cartilage and bone, there is an almost immediate appearance of deep-blue staining granules about many of the bony trabeculae. Particularly prominent are aggregations close to the cartilage-shaft junction. These blue pigment granules are concentrated about the nuclei of osteoblasts and the few osteoclasts which may be present. The development of the color is, as noted, extremely rapid and is accelerated if a stream of oxygen is played over the slice. This intense coloration appears often before any very prominent blueing can be observed in striated muscle fibers which may be present. The periosteal cells exhibit a bluish haze but the intensity or rapid appearance of color seen in osteoblasts is not found in this region. Later bluish granules can be found in marrow

cells and, later still, a few small granules can be detected in osteocytes, that is, bone corpuscles. During the course of the observation, however, no change of color can be detected in the cells or intercellular matrix material of the cartilage. Fat globules present in the marrow display a reddish-purple tint and this color, which is quite unlike the deep indophenol blue, is also seen in some of the immature cartilage cells. The exact chemical reaction in the lipid has not been pointedly studied save to say that it is not elicited by either of the two reagents alone.

The development of any blue color in the slice is easily prevented by cyanide. Such inhibition is not an absolute phenomenon since, as Friedenwald and Stiehler (19) have pointed out, a positive reaction may develop after a lag and may be prevented from developing further by the addition of more cyanide. If ferricyanide is added to the washed slice at the completion of the reaction, a faint bluish tint appears in the cartilage as well as the bony trabeculae indicating that the reagents have penetrated these tissues.

The significance of the presence of cytochrome oxidase in the osteoblast is not clear at present nor can its possible relation to osteoid formation or bone salt deposition be even hazarded. It is interesting that, in contrast, no activity is found in cartilage. Our observations have confirmed and amplified those of others (5, 35) in this latter respect.

*Succinic Dehydrogenase* An histochemical method for the demonstration of succinic dehydrogenase has been reported by Semenoff (58) who applied the well known Thunberg technique to tissue sections. We have utilized this method, with certain controls, in studying free hand slices of cartilage and bone from rats and guinea pigs.

Slices are placed in a solution of Krebs-Ringer phosphate (pH 7.4) solution containing methylene blue (1-2000). They are allowed to remain in this medium 3 or 4 minutes or longer, removed, gently blotted and placed in a small pool of 1 M sodium succinate ringed by vaseline which has been extruded from a syringe through a large bore hypodermic needle. The fluid is then covered by a cover slip which is gently pressed onto the vaseline to produce an air-tight seal, care being taken not to entrap any air bubbles in the chamber thus formed. Two control preparations are set up: in one, Krebs-Ringer phosphate solution replaces the succinate substrate, while the second control slice is placed

in equal parts of 1 M sodium succinate and 1 M sodium malonate. All preparations are then incubated on a warm plate near the microscope.

Initially the entire section is colored blue, the nuclei more intensely than the cytoplasm of the cells and ground substance of the cartilage, which is usually metachromatic. The first change to take place is a fading of color in the cartilage so that a yellow band of tissue appears above the shaft and beneath the center of ossification. In preparations with just the proper degree of staining by methylene blue this decolorization precedes any loss of color in the underlying bone and marrow. We have not been able to detect any differences in the rate of decolorization of various regions of growing cartilage. Loss of color soon appears in the bone and marrow cells as well, but this change usually follows the decolorization of the cartilage. Control sections in the Krebs-Ringer phosphate medium ultimately become colorless, but at a slower rate, while those exposed to malonate show no decolorization. As a further control, the cover slip is removed, the transformation of the colorless leucomethylene blue to methylene blue may be observed as the tissue regains its original hue.

There have been several reports on the dehydrogenase activity of cartilage. Kuwabara (35) was apparently the first to apply the Thunberg technique to this tissue in the test tube. More specifically, Rosenthal et al (52) have demonstrated succinic dehydrogenase activity in bovine articular cartilage. The histochemical studies reported above would confirm these findings although they are disappointing in that they fail to show any differences in enzyme activity in various regions of the cartilage.

*Citric Acid Dehydrogenase* The importance of citric acid in the metabolism of bone stems from several lines of investigation. The effects of citrate on the healing of rickets was noted a number of years ago (61), at the present time the exact mechanism of this phenomenon is not clear but it appears as though more is concerned than a mere increase in the absorption of calcium from the intestinal tract as a result of the presence of citrate. The presence of rather large quantities of citric acid in bone and to a lesser extent in cartilage was demonstrated by Dickens (9). It seemed therefore of interest to determine whether cartilage displayed any dehydrogenase activity when placed in a substrate of citrate.

A technique similar to that described above for succinic dehydrogenase has been applied to study citric acid dehydrogenase activity. The only difference is the use of a substrate consisting of 1 M sodium citrate. Free hand sections are placed in this medium with methylene blue buffered at pH 7.4 and observed anaerobically. There is a fairly prompt disappearance of color from the cartilage before any color change is found in control slices observed in methylene blue-buffer mixture alone. It is thus apparent that cartilage contains a system capable of dehydrogenating citric acid.

*Phosphatase* Since Robison's (49) demonstration of alkaline phosphatase in bone 25 years ago, this enzyme has been the focus of a storm of controversy over its relationship to the mechanism of bone formation. Robison (50) advanced the hypothesis that the deposition of bone salt in the organic matrix of cartilage involves the hydrolysis of an organic phosphate ester by phosphatase, thus leading to a precipitation of calcium phosphate in the tissue. The well known observations that serum and bone phosphatase values are elevated when there is an acceleration of new bone formation and depressed when osteoblastic activity is reduced, as in scurvy (24, 62) would make it seem likely that this enzyme has an important role in the calcification mechanism of bone and cartilage.

The development of an histochemical technique for the demonstration of alkaline phosphatase by Gomori (22) and Takamatsu (63) in 1939 has proved a means whereby phosphatase can be more precisely localized in tissues. In embryonic bone of chick (46) or rat (32, 34) enzymatic activity is found in the undifferentiated mesenchymal cells which give rise to cartilage. In developing bone, periosteal cells, as well as osteoblasts, display a prominent phosphatase reaction (17, 34). In addition, there is histochemical evidence of enzymatic activity in the hypertrophic cartilage cells as well as in the matrix substance between them but not in cartilage matrix elsewhere (23, 39). In contrast, bone matrix exhibits no phosphatase activity, while, as already noted, the osteoblast and, to a lesser degree, the osteocyte give positive reactions.

Our own studies have confirmed these observations. We have had difficulty, however, in using the technique advocated by Gomori (23) because of the presence of bone and have therefore resorted to studying serial sections one of which is incubated in the presence of glycerol



phosphate After a suitable interval both are stained with silver nitrate and then compared In our opinion the phosphatase reaction applied to bone is not a particularly suitable method since the duration of incubation, amount of washing etc change the end result We have therefore turned to quantitative chemical analyses which yield more precise data (15)

#### GLYCOGEN

Since the demonstration of glycogen (*zoamylase*) in cartilage by Charles Rouget in 1859 (55), a great many histochemical observations of this material have been reported With time there has grown a feeling that the presence of glycogen might have some bearing on the calcification process For instance, in 1885 Marchand (41) called attention to the increase in size of the cartilage cells at the growing ends of bones and ascribed this hypertrophy to an accumulation of glycogen in the cells In 1928 Hoffmann et al (31) stated that "a striking relationship exists between the disappearance of glycogen in cartilage and its ossification" A few years later Harris (27) also suggested that the presence of glycogen might have some bearing on the calcification process, possibly being related to Robison's (50) phosphatase mechanism More recently the presence of glycogen in cartilage cells has assumed further importance in regards to the phosphorylase mechanism of Gutman (25, 26) Several other recent studies (21, 32) have confirmed and somewhat amplified the previous investigations

Harris (28) made the pointed statement that "osteoblasts and vascular bone contain no glycogen" However, Gendre (20) seems to have noted glycogen in osteoblasts and osteocytes although his descriptions are extremely brief and not very clear

In our own studies we have utilized the Bauer-Feulgen (1) and McManus (43) techniques for the demonstration of glycogen The fixation has been absolute alcohol or absolute alcohol-picric acid-formol as recommended by Rossman (54) If silver nitrate is to be used to demonstrate lime salts, the former should, of course, be employed Glycogen was differentiated from other materials, particularly the ground substance of cartilage by the use of control sections exposed to saliva The presence of glycogen in the cartilage cell has been confirmed Glycogen is found in increasing amounts as the cartilage cell matures

In young and most undifferentiated cells only a few small pink or reddish granules can be found. With multiplication of the cells and as they increase in size, more numerous and larger granules of glycogen appear, until, in the hypertrophic cells close to the cartilage-bone junction, the cytoplasm is filled with homogeneous reddish-staining material. No glycogen can be found in the cell nuclei at any time. The most mature cells, that is those closest to the shaft and nearest the invading blood vessels, do not appear to contain glycogen. It is this area that we have observed most carefully, realizing the importance which the disappearance of glycogen may have on current concepts (25, 26) of the calcification mechanism in cartilage. We have, therefore, studied sections stained for glycogen and similar ones exposed to silver nitrate in order to bring out the exact site of lime salt deposition (a pointed comparison which to our knowledge has not heretofore been carried out). From our observations it appears that there is a rough inverse relationship to the presence of glycogen and presence of lime salt deposition in the zone of provisional calcification. In cartilaginous matrix where positive silver staining is found little or no glycogen is present in the 2 or 3 adjacent cells. The next few cells above usually contain large quantities of glycogen together with a spotty deposition of silver in the adjacent matrix. For an absolute answer to this question it would seem desirable to apply quantitative chemical methods in order to determine the relationships between the concentrations of glycogen and calcium and phosphate. Such studies are now in progress (16). Small amounts of glycogen have been observed in osteoblasts and osteocytes.

#### MUCOPOLYSACCHARIDES

Many cells and tissues, among them cartilage, have long been known to exhibit metachromasia, that is, when stained with a specific group of dyes, one of which is toluidine blue, they exhibit not the expected blue (orthochromatic) color of the dye but a different (metachromatic) tint. In the case of cartilage the matrix substance is reddish-purple (metachromatic). To offer an explanation for this phenomenon one has to consider the properties of both the tissue and the dye. In the case of the former, most cells and tissues exhibiting metachromasia contain polymerized esters of sulfuric acid and a carbohydrate (38). Dyes

exhibiting metachromasia are apparently polymerized, especially in concentrated solutions (44, 45) In such a state they change color, this, of course, may be demonstrated by measuring the absorption maxima at various wave lengths The cells and tissues capable of exhibiting metachromasia seem to have the ability to adsorb the dye, hence increasing its polymerization, as a result a different color is observed in such areas, indicating the presence of mucopolysaccharide

Cartilage, of course, contains large quantities of chondroitin sulfuric acid bound to protein The former may be separated and gives rise to sulfuric acid and chondroitin, which latter moiety yields a galactosamine, glucuronic acid and acetic acid As already noted, cartilage, at least the matrix substance and cytoplasm of hypertrophic cells, is extremely metachromatic This property has been the subject of several studies of which those from Wislocki's laboratory (7, 8, 64) are most interesting These investigators have extended observations on the well known metachromatic properties of cartilage by observing the effect of varying the hydrogen ion concentration on the staining of cartilage sections by methylene blue or toluidine blue It was found that in acid media (pH 1.5-3.0) only the cartilage cell capsules exhibited any metachromatic staining, as the environment became less acid (pH 5.0-7.0) the matrix also took on this staining property The Boston group has further noted that cartilage matrix gives a positive color with the Bauer stain a property which is not destroyed by saliva (64) In addition, metachromasia of cartilage matrix of fixed tissue is not destroyed by hyaluronidase derived from testis (8) in the concentrations used

We have repeated and confirmed these observations, employing similar techniques and have included the periodic acid method of McManus (43) which has been shown to be a specific method for demonstrating certain polysaccharides (33) Our observations have lead to little that is new save for one extremely interesting finding osteoid, like cartilage matrix, is metachromatic We have noted very thin metachromatic bands along the inner margin of the cortex in undecalcified sections That such bands are osteoid has been amply confirmed in studies on rachitic animals which will be reported elsewhere (16) Another observation of probable significance is the prominent metachromasia of the fibrillar structure of cartilage and bone

matrix In suitably stained preparations there is a definite concentration of dye along these fibrils with lighter zones in between

#### NUCLEOPROTEINS

Little information is available on the nucleoproteins of cartilage and bone Chemical analyses of the nucleoprotein phosphorus of embryo and adult sheep cartilage have revealed definite though small amounts, greater quantities are found the younger the tissue (6) The concentrations of nucleoprotein in cartilage appear to be among the lowest of any tissues thus far examined In order to obtain further information we have applied certain histochemical procedures to undecalcified sections of cartilage and bone in order to differentiate the two types of nucleoprotein, those containing desoxyribose nucleic acid and those containing ribose nucleic acid

*Desoxyribose nucleic acid* For the demonstration of desoxyribose (thymus) nucleic acid the Feulgen (12) reaction is customarily employed As is well known, this technique consists of hydrolysis of the nucleo-protein with warm normal hydrochloric acid in order to free the aldehyde group of the desoxyribose sugar which is then identified by the Schiff reagent

When this procedure is applied to undecalcified sections of cartilage and bone only the nuclei of cells (cartilage, bone and marrow) stain There is no coloration of the cytoplasm of cartilage cells or matrix of cartilage and bone The identity of the Feulgen-positive material has been further confirmed by incubation of the section in the presence of gelatin and magnesium sulfate in Veronal buffer (42) with a desoxyribonuclease prepared from calf thymus and kindly furnished by Dr Norman Weissman Following such treatment the Feulgen positive areas noted above fail to stain

*Ribose nucleic acid* Since the initial observation of Brachet (4) in 1940 a number of experiments have been carried out utilizing the effect of ribonuclease to destroy the affinity of cells for basic dyes such as methylene blue or hematoxylin

We have used formalin fixed or frozen dried tissue stained with methylene blue or hematoxylin Some of the sections were incubated with a ribose nuclease prepared from pancreas and kindly supplied by Dr Norman Weissman Sections were incubated with this prepara-

tion in acetate buffer at pH 5.0. No effect was found on the basophils in the cartilage matrix, this confirms observations reported by Dempsey and Singer (8) in tracheal cartilage.

#### LIPOID

*Neutral Fat* Leydig (37) was apparently one of the first to mention and illustrate (page 33, Fig. 16B) fat droplets in cartilage cells. A number of observers (2, 57, 59) have studied the distribution of neutral fat in the cartilage cells of various animals. There is agreement that the presence of sudanophilic material is not evidence for any "degenerative" change in the cartilage cell since one can follow a definite cycle in the appearance, accumulation and disappearance of fat, this coincides with the growth and maturation of the cartilage cell. The fat, however, disappears as the stage of hypertrophy of the cells is reached.

Our own observations have confirmed those already reported in the literature. Fat globules stained with Sudan IV are absent from perichondrial cells, as these most immature cells become larger, small sudanophilic droplets in the cytoplasm coalesce to form larger ones. In the proliferating cartilage just after the formation of rows, fat reaches its greatest prominence. As the cells increase in size and approach the zone of provisional calcification fat droplets have completely disappeared from the cells. Although the simultaneous identification of fat and glycogen in our material has not been possible one gets the impression that the former precedes the latter in maximum accumulation in the cytoplasm as the cell matures.

#### HYDROGEN ION CONCENTRATION

Not a great deal is known concerning the hydrogen ion concentration of cartilage and bone, although local changes are often invoked to explain the calcification mechanism. One of the earliest series of observations was performed by Rous (56) who injected litmus as well as other dyes into animals. The following changes were found: *litmus* (red at pH 6.2, blue at pH 8.4), cartilage matrix "sky-blue", while bone was deeper blue, bromocresyl green (yellow at pH 4.0, blue at pH 6.0), cartilage, blue, chlorphenyl red (yellow at 5.3, purple at pH 6.2), cartilage and bone, rose purple, bromocresyl purple (yellow at pH 5.4, blue at pH 7.0), cartilage matrix, purplish blue, bone, deep

blue, cresol red (yellow at pH 7.2, red at pH 8.8), cartilage, yellow Dempsey et al (7) have noted that cartilage gives a red-orange color with neutral red (red at pH 6.8, yellow at pH 8.0) Pierce (48) used the gunhydrone electrode to measure the pH of normal resting and proliferating cartilage cells and found values of 7.35 and 7.39 respectively.

We have applied a series of indicator dyes to fresh slices of cartilage and bone in order to determine whether any information could be obtained as to the approximate pH of the tissue. The results are summarized in Table 1. It will be seen from this that cartilage has an over-

TABLE I

INDICATOR	pH RANGE	COLOR CHANGE	COLOR OF CARTILAGE
Bromphenol blue	3.0-4.6	Yellow-blue	Blue
Bromcresyl green	4.0-5.6	Yellow-blue	Blue
Methyl red	4.2-6.3	Red-yellow	Yellow
Litmus	4.5-8.3	Red blue	Blue
Chlorphenyl red	5.0-6.6	Yellow-red	Red
Bromcresyl purple	5.4-7.0	Yellow-purple	Purple
Bromthymol blue	6.0-7.6	Yellow-blue	Blue
Phenol red	6.6-8.2	Yellow-red	Orange
Neutral red	6.8-8.0	Red yellow	Orange
Cresyl red	7.2-8.8	Yellow-red	Yellow
Phenolphthalein	8.3-10.0	Colorless-red	Colorless

all pH in the range of pH 7.4. Perhaps the most significant finding is a negative one—that the pH of cartilage is not alkaline.

We have also applied a series of oxidation-reduction indicators of known potential to fresh slices of cartilage and bone in an attempt to detect differences in potential. The results have been difficult to interpret and will not be commented upon at this time.

#### DISCUSSION

There are certain important aspects of the metabolism of cartilage and bone with relation to growth processes which have been reported but have not been mentioned in what has gone before. It would seem appropriate to call attention to these, point out certain integrations and suggest further directions for study.

It is apparent that a beginning has been made in our understanding of the biochemistry of cartilage. A number of observations, many admittedly unrelated, have been recorded. The problem has been approached from several standpoints: interest in exploring the metabolism of a virtually avascular tissue such as cartilage, an attempt to obtain information which might throw some light on the arthritis problem, and the hope of elucidating the calcification mechanism.

At the outset it should be pointed out that a variety of animals have been used, that the age of the animals have varied from group to group and that articular cartilage, which would appear to be a most sluggish tissue, has been most commonly employed in the studies reported. However, it has been shown that cartilage has a definite, though low  $QO_2$  (10, 30, 40, 51), this is related to the age of the animal (53) and more important to the site from which the specimen for study is taken, rate of growth playing an important role (10, 53). In addition it has been found that cartilage has aerobic and anaerobic glycolysis (5, 11, 35, 51), the latter being more marked. Methylene blue has a markedly stimulating effect on metabolism, increasing the  $QO_2$  of cartilage many times (5, 30, 52). This observation has led to a study of dehydrogenase systems in cartilage and it would appear that enzymes capable of dehydrogenating glucose (52), mannose (52), succinate (52), lactate (52) and pyruvate (52) are present. In addition, the presence of glycogen has, of course, aroused a great deal of interest, this phase will be dealt with shortly. Glycogen was early shown to break down into lactic acid (31). Cartilage appears to metabolize certain hexose-phosphate compounds (40). The possible role of these with especial reference to the presence of a phosphorylase (25, 26) which would aid in the local mobilization of phosphate is doubtless of importance. The demonstration of a citric acid dehydrogenase in cartilage referred to above may also be of some significance because of the presence of this material in cartilage (9) and its effect on the healing of rickets (61). Phosphatase which has been the center of such a controversial storm for many years will be mentioned more in detail below. Our failure to find cytochrome oxidase activity in cartilage confirms brief references to similar findings in the literature (5, 35). From the above observations one can hardly look upon cartilage as a metabolically inert material. To be sure articular cartilage is much more sluggish than

cartilage cells which are proliferating. It is hoped that more attention will be focused on the latter type of tissue with special emphasis on its metabolism with respect to bone formation.

When we consider the biochemistry of bone itself the void is virtually complete. Furthermore it would seem that there is little approach in the way of orthodox biochemical procedures due to the presence of marrow cells. Histochemical studies would seem to be extremely important here. The presence of cytochrome oxidase in osteoblasts has been referred to, though its significance in these cells is not at all clear. Phosphatase activity can also be visualized in osteoblasts in which cells glycogen is also present. The organic portions of bone may be studied histochemically as well, at least to demonstrate polysaccharide moieties (29, 64). An approach to the biochemistry of the osteoblast might be made through the use of periosteal cells which can be stripped from the bone and thus provide a more or less pure culture of osteoid forming material. Such an approach has already provided quantitative data regarding phosphatase activity of periosteum (15).

At the present time certain points have been established regarding the possible mechanism of the calcification process in cartilage matrix. The dynamics of the phenomenon in osteoid (bone matrix) is less clear.

From the data which are available it seems fairly clear that both humoral and local concentrations of inorganic materials are extremely important. This role of the former in the pathogenesis of rickets need only be mentioned since the prompt deposition of lime salts in the intact organism as well as in cartilage slices *in vitro* (60) is too familiar to warrant further discussion. Local factors responsible for the deposition of inorganic salts have been concerned with mechanisms for increasing phosphate concentration. Little recent attention has been paid to the deposition of calcium or carbonate, save that the possible role of carbonic anhydrase in the latter's appearance has been suggested (3). The story of phosphate deposition began with the demonstration of the enzyme alkaline phosphatase by Robison (49). This led to a tremendous amount of work on this enzyme and possible substrates for it, culminating in the conjecture by Harris (49) that glycogen might be related in some way to the phosphatase mechanism. With the gradual evolution of our knowledge of the glycogenolytic cycle such a con-



cept seems even more plausible, particularly too, since at least one enzyme, phosphorylase, which changes glycogen to hexosephosphate has been identified by Gutman and his co-workers (25, 26) in epiphyseal cartilage. No other enzymes concerned in the breakdown of glycogen have as yet been identified nor, of course, has phosphorylase activity been precisely localized at the site of glycogen breakdown. Currently, then, glycogen is thought to be the beginning material for a series of reactions which would yield a phosphate ester from which phosphatase would liberate free phosphate. It is of further interest that one of us, in association with Dr M D Levine (36) has demonstrated the presence of a lecithinase in cartilage which will break down lecithin into a diglyceride and phosphocholine, the latter is assumed to be broken down by alkaline phosphatase. It would seem then that there are at least two unrelated mechanisms for providing an increased concentration of phosphate in the zone of provisional calcification of cartilage.

Little save conjecture is known of the local or tissue factors which must also be important in the calcification mechanism. The possible relationship of matrix to calcium deposition was first mentioned 40 years ago by Pfaundler (47) and later studied by Freudenberg and Gyorgy (18). It has seemed of interest to us that bone matrix and cartilage matrix exhibit the same staining reactions with the metachromatic dye, toluidine blue and the periodic acid-leuco fuchsin reaction. This may indicate that chemically these two structures are closely related.

The basophilia of cartilage is doubtless due to the presence of chondroitin-sulfuric acid. We have not as yet carried out any experiments designed to abolish this basophilia save to demonstrate that it is not destroyed by two types of nucleoproteinases.

#### SUMMARY

A study has been carried out on normal cartilage and bone applying certain histochemical techniques in an attempt to gain a base from which to study certain diseases. The results of these techniques are presented and discussed.

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## PLATE 1

### Explanation of Figures

FIGURE 1 Free hand slice of rat bone treated with the nadi reagent. There are accumulations of pigment about osteoblasts whose nuclei do not stain.

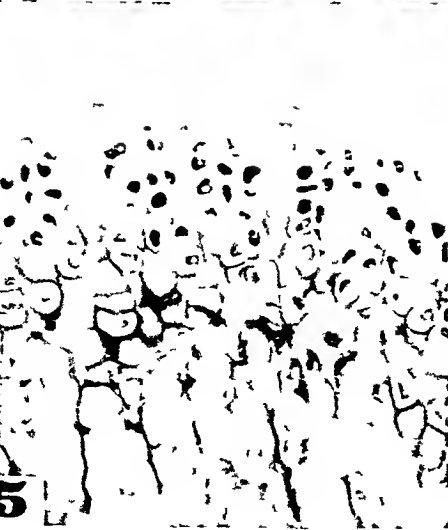
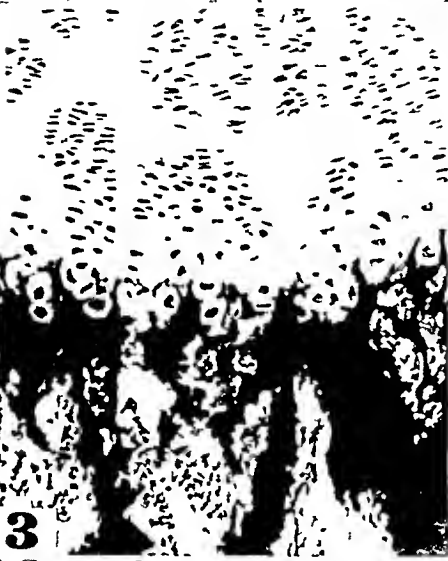
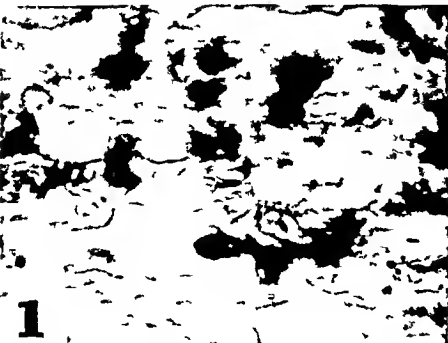
FIGURE 2 Higher power of free hand slice similar to Figure 1.

FIGURE 3 Section of undecalcified normal rat rib stained with 2% silver nitrate.

FIGURE 4 Similar section incubated with buffered sodium glycerophosphate for 2 hours and then stained with silver nitrate. Note increase in silver staining in cartilage matrix and hypertrophic cells. The marrow cells also give a positive reaction.

FIGURE 5 Section stained with the periodic acid-leucofuchsin technique. The dark staining material in the hypertrophic cartilage cells is glycogen.

FIGURE 6 Frozen section of human costal cartilage stained with Sudan IV to demonstrate fat globules which stain more darkly than the cell nuclei.





# ACUTE HEPATITIS IN GENERAL HOSPITAL PRACTICE<sup>1</sup>

## THE RÔLE OF TRANSFUSIONS AND INOCULATIONS

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The transfusion of whole blood or plasma has been employed with increasing frequency in the treatment of a wide variety of disease states. At the same time, impressive evidence has accumulated that this procedure is not without risk. Among these risks, the appearance of hepatitis some time after the transfusion of blood has been described repeatedly in recent years. This syndrome, usually called homologous serum jaundice, has been observed after the injection of whole blood, plasma or serum, and after the use of needles and syringes contaminated with unsterilized blood or serum. During the last few years, the number of blood and plasma transfusions given at The Johns Hopkins Hospital has risen sharply. It seemed of interest, therefore, to try to estimate the extent of the problem presented by homologous serum jaundice in hospital practice. The data to be presented emphasize the fact that, at present, homologous serum jaundice and acute infectious hepatitis cannot be distinguished on clinical grounds.

(1) *Description of the Case Material* The histories were reviewed of all cases of hepatitis due to any cause in patients who were hospitalized at The Johns Hopkins Hospital between January 1, 1937 and December 31, 1948. Those patients with hemolytic or obstructive jaundice, amebic hepatitis, Weil's disease, hepatitis due to sulfonamides or cinchophen, or cirrhosis of the liver were eliminated from consideration. There remained 287 patients with acute infectious hepatitis (or catarrhal jaundice), acute yellow atrophy of the liver, post-arsphenamine hepatitis, and homologous serum jaundice (Table I).

Which cases one should call homologous serum jaundice cannot be determined with certainty. Different authors give different estimates for the incubation period of this disease. These vary from about 2

<sup>1</sup> These studies were conducted under contract with the office of Naval Research, U S Navy.

weeks to 7 months (1-4) In the analysis which follows, any instance of hepatitis is called homologous serum jaundice if it appeared between 5 weeks and 5 months after the injection of human blood or its products It is obvious that naturally occurring acute infectious hepatitis may have appeared in some patients following transfusion, and it is therefore impossible in any given instance to be certain of the route of infection

Moreover, a review of the clinical and pathologic features of the cases of hepatitis studied demonstrated that they could not be satisfactorily differentiated Although the cases designated as homologous

TABLE I  
*The Relative Frequency and Mortality of Various Types of Hepatitis*

TYPE OF CASE	NO	TOTAL	NO FATAL CASES	CASE FATALITY
		%		%
Hepatitis after Injection of Homologous Blood or Blood Product	40	14 0	11	27 5
Hepatitis after Injection of Arsenical Compounds*	51	17 8	6	11 8
Hepatitis after Insertion of Other Needles†	39	13 6	2	5 1
Hepatitis during Pregnancy	22	7 7	2	9 1
All other Acute Hepatitis	148	51 5	12	8 1
Total Cases, Excluding Duplications	287		32	11 1

\* Including 10 non-fatal cases treated with arsenicals during pregnancy

† Including 2 non-fatal and 1 fatal case after insertion of needles during pregnancy

serum jaundice were more severe than the average, no other essential difference was noted In the group considered, regardless of the designation of the illness, most patients presented a relatively benign picture of abdominal complaints followed after several days by jaundice, light colored stools, and dark urine Examination in the hospital disclosed that all the patients were jaundiced Frequently the liver was palpable, and occasionally the spleen was as well Ordinarily the process gradually subsided Occasionally, however the hepatitis was more severe, and 32 patients, 11 2 per cent of the total, died The high mortality rate may have been due in part to the fact that only the more obviously ill patients with hepatitis were admitted to the hospital during most of the period of this study

Acute hepatitis was observed in 22 pregnant women There was no

particular relationship between the time of onset of the hepatitis its severity, and the stage of pregnancy. Five of these patients aborted during their illness including 2 patients who died and 2 others who were under treatment for syphilis with arsenical compounds. Five other patients had premature deliveries during the course of their illness.

TABLE II  
*Laboratory Studies in Patients with Hepatitis*

DIAGNOSIS		WBC CELLS PER MM. <sup>3</sup>			SR MM/HR			ALK. PHOS- PHATASE (B.C.)		C.T.				I.T.		
		< 10 000	10-12 000	> 12 000	< 10	10-20	> 20	0-12 0	> 12 0	0-1	1-2	2-3	3-4	< 6	6-9 0	> 10
Hepatitis after Injec- tion of Homologous Blood or Blood Product	Non fatal cases	19	6	3	4	3	5	19	6	7	10	2	0	0	0	11
	Fatal cases	5	2	4	0	1	0	5	1	0	6	0	0	0	0	3
Hepatitis after Inser- tion of Other Nee- dles																
Arsenical	Non fatal cases	35*	2	6†	6	14	13*	10‡	10‡	2	4	1	0	0	0	0
	Fatal cases..	2	2	2	0	1	1	1	1	0	0	0	0	0	0	0
Non Arsenical	Non fatal cases	23	4	2	5	7	8	20	3	4	8	1	1	1	1	9
	Fatal cases	1‡	0	1	0	0	2‡	1‡	0	0	0	0	0	0	0	0
Hepatitis During Pregnancy (total)	Non fatal cases	10	1	7	0	0	3	6	2	1	0	0	0	0	0	1
	Fatal cases	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0
All other Acute Hep- atitis	Non fatal cases	110	10	13	12	41	40	59	11	5	33	1	1	1	19	
	Fatal cases	6	1	5	3	1	1	3	1	0	2	0	0	0	0	2
Total Excluding Du- plications	Non fatal cases	195	23	23	27	65	62	110	30	19	55	5	2	4	42	
	Fatal cases	14	5	13	3	3	4	10	3	0	8	0	0	0	5	

\* Including 6 pregnant women

† Including 3 pregnant women.

‡ Including 4 pregnant women

§ Including 2 pregnant women

|| Including 1 pregnant woman.

In one of these 5 cases, the baby died 8 hours after birth, there was no evidence of hepatitis in the fetal liver. The case fatality rate of hepatitis was the same in pregnant as in non-pregnant patients.

Laboratory studies did not help to differentiate the patients with acute hepatitis (Table II). In this series, bilirubinemia was invariably present. The sedimentation rate measured by the Wintrobe method was elevated above 20 mm per hour in 41 per cent of the patients.



tested, and was no more frequently elevated in fatal than in non-fatal cases. The white blood cell count was less than 10,000 per mm<sup>3</sup> in 75.1 per cent of the patients with acute hepatitis, between 10,000 and 12,000 in 10.0 per cent, and above 12,000 in 14.7 per cent. In many of these patients, extraneous factors may have been responsible for the leukocytosis. Leukocytosis was present more frequently in pregnant patients and in patients who died. The alkaline phosphatase activity of the serum was 12 Bodansky units or less in 120 of 153 patients in whom it was determined. It was not elevated above 12 Bodansky units any more frequently in fatal than in non-fatal cases. However, it is noteworthy that the alkaline phosphatase activity of the serum was more than 12 Bodansky units in 11 of 22 patients with post-arsphenamine jaundice, although none of these patients had erythema of the ninth day (5). The cephalin cholesterol flocculation test was positive, 2 plus or higher, in 63 of 82 patients, including all of 8 patients who died. The thymol turbidity was 10 Maclagan units or higher in 45 of 52 patients tested, and between 6 and 10 units in 2 of the others. In each of the 5 fatal cases tested, the thymol turbidity was higher than 10 units.

The liver was examined at autopsy in 28 of 32 fatal cases, and by biopsy in 7 non-fatal cases. No features were described which distinguished between acute infectious hepatitis, homologous serum jaundice, or post-arsphenamine hepatitis. When the destruction of the hepatic parenchyma was particularly severe, the diagnosis of acute yellow atrophy was often made, but the etiology of the lesion could not be determined pathologically.

(2) *The Incidence of Previous Injections of Human Blood in Patients with Acute Hepatitis*. During the period of this study, 40 patients were hospitalized with hepatitis in whom the first symptom appeared 5 weeks to 5 months after the injection of human blood or a fraction of human blood. Twenty-eight of these 40 patients had been injected with whole blood, 8 with whole blood and plasma, 2 with plasma alone, one with yellow fever vaccine from a known icterogenic lot (6), and one with human globulin. In other words, one out of every 7 patients hospitalized with hepatitis had been injected with human blood or a blood product 5 weeks to 5 months before the onset of the hepatitis. Only 20 of these patients were admitted during the first 10 years covered by this study, whereas an additional 20 were hospitalized

during the last 2 years. This increased incidence may reflect the more common therapeutic use of blood and its products in the last few years, since the number of patients with hepatitis of all types admitted annually throughout the study remained approximately constant.

The hepatitis which followed injection of human blood was often severe, and 11, 27.5 per cent of the 40 patients with this history, died. On the other hand only 21, or 8.5 per cent of the remaining patients died.

(3) *The Incidence of Parenteral Injections Other than Blood in Patients With Acute Hepatitis*. The transmission of hepatitis through the use of contaminated syringes and needles has been described repeatedly (7-13). There were 247 patients with acute hepatitis who had not previously had an injection of human blood. Ninety of these patients were known to have had a parenteral test or parenteral injection 5 weeks to 5 months before the onset of their disease. The nature of the histories reviewed was such that it is likely that more of the cases of hepatitis may have followed parenteral inoculation. Fifty-one of the 90 patients were under treatment with arsphenamine, neoarsphenamine, tryparsamide, or mapharsen for syphilis. Arsenic is a known hepatotoxic agent, and some or all of the patients under treatment with arsenical compounds may have had a true arsenical hepatitis. However, it is of note that 13 of these 51 patients were subsequently treated with mapharsen without a single instance of recurrent hepatitis. There remained 39 patients whose hepatitis followed 5 weeks to 5 months after a parenteral test or injection other than arsenic.

The meaning of these data depends upon a knowledge of the frequency of a history of parenteral tests or injections in patients hospitalized for causes other than hepatitis. For this reason, an estimate was made of how frequently control patients without hepatitis gave a history of parenteral tests or injections before they became ill. The case histories of 287 patients without hepatitis, admitted to The Johns Hopkins Hospital during the period of this study, were reviewed. In each instance the control patient was the one whose admission directly followed on the same ward that of a patient with hepatitis. In this manner an equal number of controls was assembled, similar to the patients with hepatitis in respect to age, sex, race, economic status and time of admission to the hospital. Each control chart was searched for

a history of a parenteral test or injection 5 weeks to 5 months before the onset of the illness for which the patient was hospitalized. In the analysis which follows, the cases of pregnant women were eliminated from consideration in both the hepatitis and control series, since these cases were not comparable in the 2 groups. In the control series, the pregnant patients were usually admitted to the hospital in labor and had had blood studies during their pregnancy. Most of the patients with hepatitis, on the other hand, were hospitalized early during the course of their pregnancy, before any blood studies had been performed.

There remained 264 patients with hepatitis and 268 control patients. In the period between 5 weeks and 5 months before the onset of the illness for which they were hospitalized, 2 of the control patients had been transfused, 8 had been treated with arsenical compounds, and 20 of the remaining 258 patients had had a parenteral test or injection. On the other hand, 40 of the patients with hepatitis had been transfused, 41 had been injected with arsenical compounds, and 36 of the remaining 183 patients had had a parenteral test or injection. In other words, only 8 per cent of the control patients had previously had a parenteral test or injection of material other than blood or arsenic, compared with 20 per cent of the patients with hepatitis. The probability that this difference could have occurred by chance is less than one in 100. The data, therefore, suggest that there may possibly have been a causal relationship between the preceding parenteral test or injection and the hepatitis.

(4) *The Incidence of a Previous Injection of Human Blood in Patients who Died of Acute Hepatitis*. During the period of study, 32 patients with acute hepatitis, postarsphenamine hepatitis, acute yellow atrophy of the liver, or homologous serum jaundice died. Eleven of these 32 patients had had an injection of human blood, plasma, or globulin before the onset of their hepatitis.

(5) *The Incidence of Hepatitis in Patients Transfused with Blood or Plasma*. The available data do not answer the question of how frequent hepatitis was in relation to the number of blood transfusions given. Only sporadic information was available concerning the number of transfusions of blood and plasma given at the hospital before 1946. In this study, no attempt has been made to follow each patient who had been transfused to learn whether he subsequently had hepatic

tis Only those who were *hospitalized* because of their hepatitis were included in the series It has been ascertained that other patients, not included in this series but transfused at The Johns Hopkins Hospital, have been under treatment elsewhere for acute hepatitis (14) On the other hand, patients transfused elsewhere who came to The Johns Hopkins Hospital with hepatitis were included in the present series Finally, it may be assumed that only the more obviously ill patients were admitted to the hospital The invariable presence of jaundice in the patients hospitalized supports this view

Approximately 2800 patients per year were transfused at The Johns Hopkins Hospital with blood, plasma, or both, in 1947 and 1948 These figures include 2100 patients who were transfused with blood, and 1000 patients with plasma During this same period, approximately 85 patients per year were hospitalized with hepatitis after blood or plasma transfusions which had been given at this hospital, and 2 per year died The proportion of patients re-admitted to The Johns Hopkins Hospital with hepatitis after transfusion, then, was one to each 330 patients transfused, and the proportion of fatalities was one to each 1400 patients transfused For the reasons outlined, the actual incidence of the disease was undoubtedly higher The relative incidence of hepatitis after transfusions of blood compared with plasma could not be determined since some of the patients received both

#### DISCUSSION

Except for the use of human volunteers, no method is available at present to study the etiology of acute infectious hepatitis For this reason it is not possible to state with certainty in any particular case that a causal relationship exists between the injection into a patient of human blood and the subsequent development of acute hepatitis None the less, it is impressive that 14.0 per cent of the patients admitted to The Johns Hopkins Hospital with acute hepatitis had been transfused between 5 weeks and 5 months before This period of time is the same as the incubation period previously reported when hepatitis followed the injection of homologous blood in human subjects (1-4) Tentatively, then, these patients may be presumed to have had homologous serum jaundice

Hepatitis after transfusion, as it was seen in the hospital, was a

serious and often fatal disease. In the present series, 27.5 per cent of these patients died. This fatality rate is comparable to that reported by others. For example, Sheinberg, Kinney, and Janeway (15) observed that 4 of 11 patients with homologous serum jaundice after transfusion died. And Snell, Wood, and Meinenberg (16) observed a case fatality rate of 19 per cent. The reported fatality rates may have been too high, since probably only the more severely ill patients were hospitalized. None the less, the disease appeared to be much more severe than the homologous serum jaundice which followed vaccination with yellow fever vaccine containing human serum (6). Very likely this difference was due to the much larger inoculum of virus which occurred during blood transfusion. It is noteworthy that the case fatality rate of hepatitis in the present series in patients who had had blood counts, venepunctures, or parenteral injections other than blood was no higher than in patients who had not received injections.

No estimate of the frequency of homologous serum jaundice was possible from the data available. However, in previously reported studies the proportion of patients transfused who subsequently developed hepatitis was high. For example, one in every 222 transfusions of blood or plasma at the Peter Bent Brigham Hospital was followed by hepatitis (15) and one in every 21 patients transfused with pooled plasma in Upper New York State developed hepatitis (17). The data suggest that homologous serum jaundice is a problem which is serious enough so that the indications for blood or plasma transfusions should be weighed carefully against the risks involved. Furthermore, the use of pooled plasma or multiple transfusions should be avoided wherever possible since the risk of transmitting homologous serum jaundice is proportional to the number of blood donors used.

It has been demonstrated repeatedly that acute hepatitis can be transmitted by minute quantities of infectious materials. A number of epidemics have been described in which hepatitis apparently resulted from the use of inadequately sterilized needles or syringes (7-13). In the present study, approximately 20 per cent of cases of hepatitis, not following blood or plasma transfusions or injections of arsenical compounds, occurred in patients who had previously had a parenteral inoculation or test. In a control group of patients, the incidence of parenteral inoculation was only 8 per cent. The evidence indicates

that injections of any variety or the withdrawal of blood should be made only with needles, syringes, or lancets which have been properly sterilized. The only practical agent known which will destroy the virus of hepatitis is heat (8). The inadvertent inoculation of patients with the virus of hepatitis may be avoided by boiling or autoclaving all needles, syringes, and lancets before their use in each patient. Sterilization with such agents as phenol (18), ether (18), and merthiolate (19) has been shown to be ineffective. It would not be sufficient to boil or autoclave the needles and syringes used on patients with hepatitis alone. There is considerable evidence either that there are carriers of the virus, or that virus is present in the blood both during the incubation period (20-22) and subsequent to the disease (23).

No satisfactory evidence has been reported as to whether infectious hepatitis and homologous serum jaundice are separate entities or variations of the same disease which depend on the route of infection (24). It is of interest that in this study the clinical, laboratory, and pathologic features of the patients with homologous serum jaundice did not differ from those of patients with acute hepatitis from other causes. The greater severity reported in post-transfusion hepatitis could well be explained by the dosage of the inoculum of virus.

The data presented indicate that acute hepatitis during pregnancy is frequently attended by abortion or premature delivery.

#### SUMMARY

(1) Approximately one out of every 7 patients hospitalized with acute hepatitis during a 12 year period had been transfused with homologous blood or a blood fraction 5 weeks to 5 months before the onset of the hepatitis. The case fatality rate in 40 patients with homologous serum jaundice was 27.5 per cent.

(2) No clinical, laboratory, or pathologic features other than differences in severity were observed which distinguished between homologous serum jaundice and other varieties of acute infectious hepatitis. Acute infectious hepatitis which occurred during pregnancy was frequently associated with abortion or premature delivery.

(3) Twenty per cent of 183 patients with hepatitis had had a parenteral test or injection of material other than blood or arsenic within 5 weeks to 5 months before the onset of their illness. Only 8 per cent of

258 control patients without hepatitis had had a similar test or injection. The probability that this difference could have occurred by chance is less than one in 100. The data emphasize the hazard of transmitting hepatitis by any procedure in which the skin is pierced, unless adequate sterilization of instruments has been achieved. All syringes, needles, and lancets, whether used for withdrawal of blood or injection of any material, should be boiled or autoclaved before their use in each patient in order to prevent the transmission of hepatitis.

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# COLONMETROGRAPHIC STUDIES OF THE EFFECTS OF SECTION OF THE PARASYMPATHETIC NERVES OF THE COLON\*

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The work of Liem (1, 2, 3, 4) and his associates on the etiology of ulcerative colitis has directed interest toward the neurogenic aspects of this disease and the rôle played therein by spasm of the colonic musculature. Recently Dennis (5) has used vagus section in treatment of patients with ulcerative colitis in an effort to reduce spasm and hypermotility of the colon and to slow the intestinal transit time.

The present investigation has been carried out in order to assess the effects of section of the various motor nerves of the colon on the tone and reactivity of the normal large intestine. For this purpose experimental observations have been made in dogs, and clinical studies have been carried out in patients undergoing vagotomy for peptic ulcer.

## THE COLONMETROGRAM

The colonmetrogram was described by White and his associates (6) in 1940 as a means of recording manometrically the tone, reflex irritability and sensitivity of the colon as a whole. The apparatus used consists of a vertical glass tube manometer connected on one side to an intravenous drip set and on the other to a 100 cc. Foley catheter (Figure 1). Water, saline or dilute barium sulfate solution may be used as the infusing fluid. The reservoir is filled with fluid at body temperature and the tubing cleared of air. The base line of the manometer is adjusted so that the meniscus of the fluid column is at the zero mark when the tip of the rectal catheter is level with the subject's anus. After inserting the tip of the catheter into the rectum the infusing fluid is permitted to run into the colon at a constant rate of 100 cc. per minute in observations on patients and 25 cc. per minute in studies

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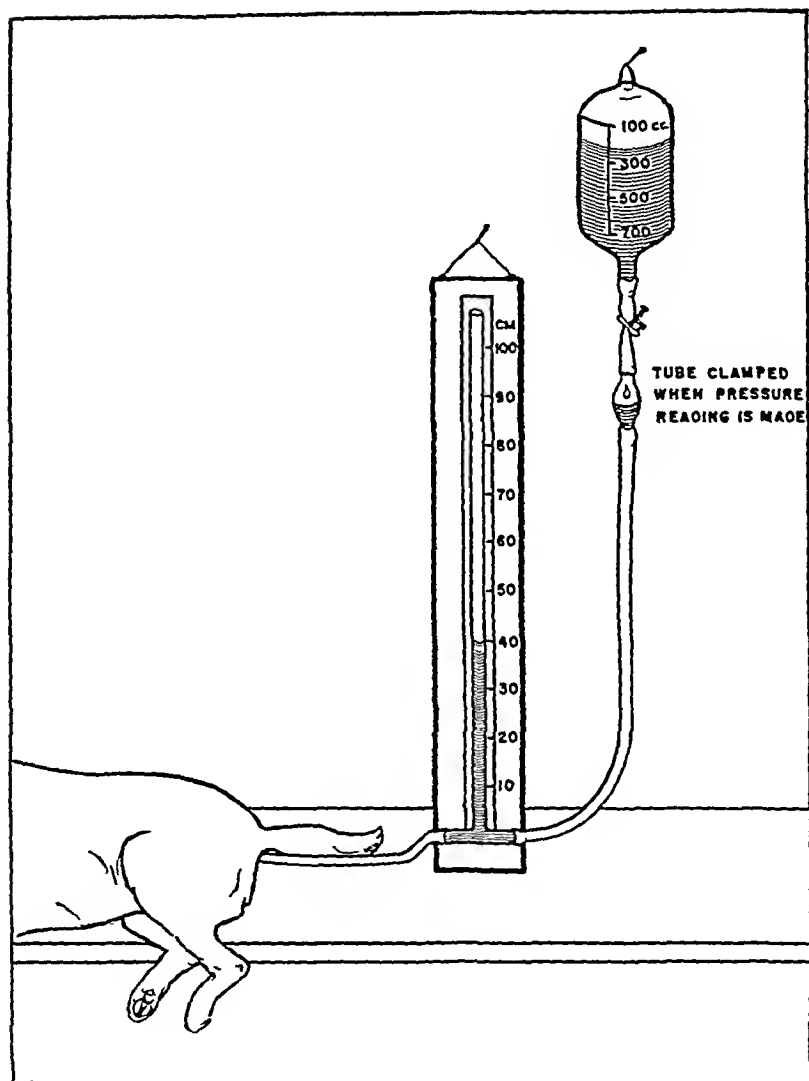


FIG 1 APPARATUS FOR COLONOMETROGRAPHIC STUDIES

on the dog's colon. The pressure recordings are then plotted against the volume of fluid introduced in graphic form.

The normal colonometrogram in man resembles the normal cystometrogram (6) except for the fact that a four-fold larger volume of fluid is

usually required for filling the colon (Figure 2) The smooth muscle in the colon, as in the bladder, reacts to stretch stimuli by reflex contraction White and his associates have demonstrated that various types of neurologic lesions are associated with changes in the colonmetrogram (as in the cystometrogram) characteristic of the level of the lesion

Colonmetry in dogs cannot be carried out satisfactorily without anesthesia—otherwise the intense voluntary straining renders the curves meaningless An intratracheal catheter prevents closure of the glottis and obviates to a large degree the diaphragmatic component of the defecation pattern Sodium pentobarbital has proven to be the

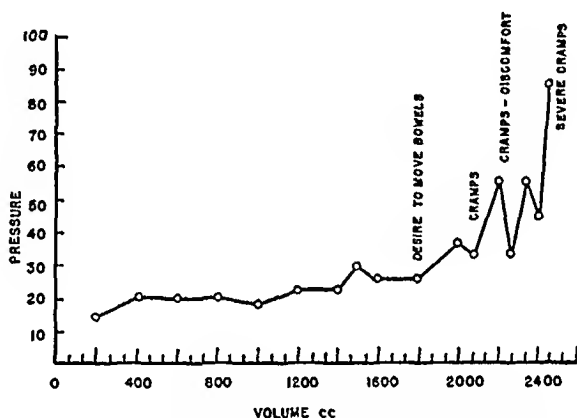


FIG 2 THE NORMAL COLONMETROGRAM IN MAN

From a patient in this study Pressure in centimeters of 0.85% saline

most satisfactory anesthetic agent from the point of view of repetition of dosage for comparative studies and minimal alteration of the reflex activity of the bowel The use of drugs such as morphine, atropine, or curare is contraindicated because of profound disturbances in colonic responses following their administration A Foley catheter is necessary to prevent leakage from the anus in dog experiments Adequate preparation of the bowel for colonmetry in the dog includes a 36 to 48 hour period of starvation and repeated cleansing enemas of tap water 12 hours before the observations are made Cathartics are to be avoided

The colonmetrogram in the normal anesthetized dog resembles

closely the normal filling curve of the colon in man except for the smaller volume required to fill the dog's colon (Figure 3) A standard dilute solution of barium sulfate (75 gms in 1 liter of water plus 5 gms acacia) used as the infusing fluid in the dog experiments permits the colonmetrogram to be carried out under fluoroscopic control Opening

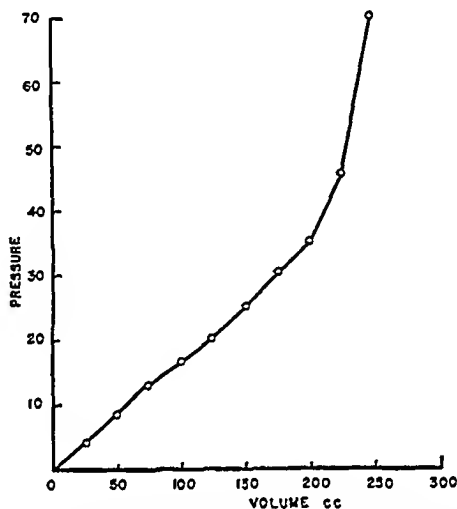


FIG 3 THE NORMAL COLONMETROGRAM IN THE DOG

Pressure in centimeters of standard dilute barium solution (75 gms  $\text{Ba SO}_4$  + 5 gms acacia in 1 liter water)

of the ileocecal valve with reflux of barium into the ileum is taken as the end-point of the filling curve in the anesthetized dog

#### METHODS

Adult mongrel dogs weighing 8 to 15 kg were used in the animal experiments All animals were anesthetized with sodium pentobarbital given intravenously in doses of 30 millograms per kilogram of body weight for each experiment Control colonmetrograms and roentgenograms of the barium-filled colon were made in each instance After obtaining satisfactory control observations each animal was given a rest period of several days and then subjected to operative interruption of one set of the various motor nerves of the colon All operations were carried out with aseptic technic, intratracheal ether

or intravenous sodium pentobarbital (30 mg /kilo of body weight) were used for anesthesia. After recovery from laparotomy or thoracotomy, colonmetrographic and roentgenographic observations were made of the colon of each animal, usually beginning at 14 days after operation and thereafter at intervals of 1 month.

TABLE 1

*Summary of Colonmetrographic Observations after Section of Nerves to Colon*

DOGS	NO. OBSERVATION	PERIOD OF STUDY	MEAN RESULT
<i>Vagus Nerves</i>			
1 C2	4	4 mos	No change
2 C4	2	1 mo	No change
3 C7	2	1 mo	No change
4 C12	4	4 mos	Slight flattening with return
5 C18	4	3 mos	No change
6 C28	3	2 mos	No change
<i>Pelvic Nerves</i>			
1 C24	3	5 mos	Flat curve
2 C29	3	2 mos	Flat curve
3 C36	3	3 mos	Flat curve
4 C37	5	9 mos	Flat curve
5 C34	2	1 mo	Flat curve
6 C2	4	9 mos	Flat curve
7 C38	3	2 mos	Flat curve
<i>Hypogastric Nerves</i>			
1 C6	6	5 mos	No change
2 C7	4	3 mos	Slight flattening with return
3 C9	5	5 mos	No change
4 C18	4	6 mos	No change

In the colonmetrograms on patients the procedure described by White was followed. No drugs were used before or during the colonmetrogram.

## RESULTS

### 1 Vagus nerves

Six dogs were used in these experiments. In each instance the vagus trunks were divided just above the diaphragm with excision of 4 cm segments from each trunk and careful extirpation of the periesophageal

vagal plexus Colonmetrograms and barium enemas were carried out in these animals following vagotomy for survival periods ranging from 1 to 4 months A total of 19 colonmetrograms were done during the period of study (Table 1) In 5 animals there was essentially no change

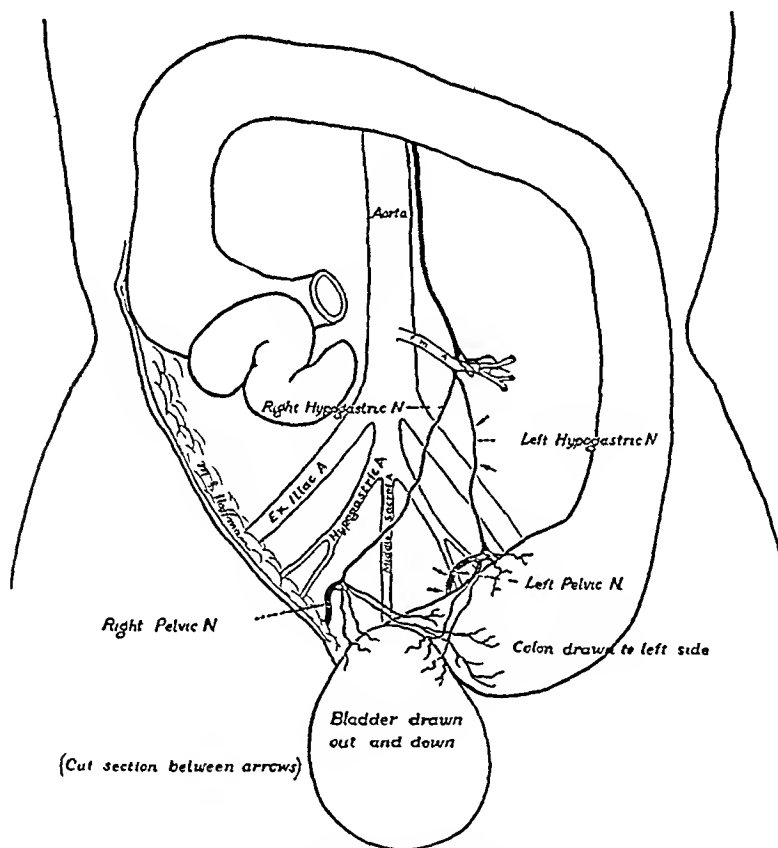


FIG 4 SKETCH DEPICTING THE HYPOGASTRIC NERVES AND THE PELVIC NERVES IN THE DOG

in the colonmetrograms following vagotomy In the remaining animal the curve obtained 2 months after operation showed some reduction in tone but subsequent curves were identical with the control colonmetrogram Roentgenograms of the barium filled colon showed no apparent change in caliber of the bowel after vagotomy in any of the animals Defecation was normal in these dogs, and no diarrhea was encountered

## 2 Pelvic nerves (*Nervi erigentes*)

Seven dogs were used in these experiments. Bilateral section of the pelvic nerve trunks was carried out in each animal as shown in Figure 4. Bladder paralysis developed promptly in all animals necessitating repeated catheterization during the post-operative period. In 3 animals a large suprapubic cannula was sutured into the bladder at the time of pelvic nerve division in order to obviate urinary retention.

Colonmetrograms and barium enemas were carried out in these animals for survival periods ranging from 1 to 9 months. A total of 23

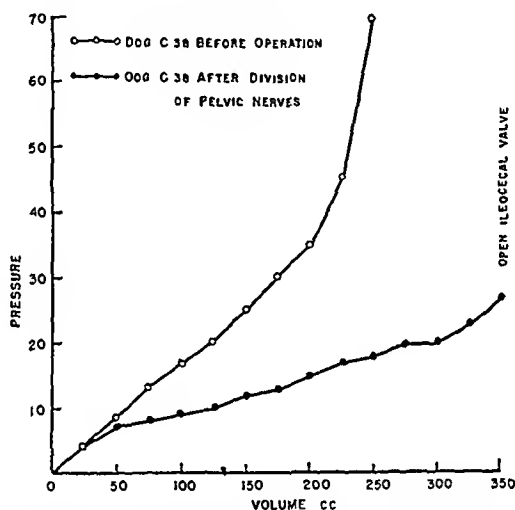


FIG 5 TYPICAL COLONMETROGRAM BEFORE AND AFTER PELVIC NERVE DIVISION IN THE DOG

colonmetrograms were done during the period of study (Table 1). Flat curves were obtained consistently in all dogs after pelvic nerve division (Figure 5). Barium enemas showed slight dilatation of the distal half of the colon in most animals, but in no instance did the dilatation reach a degree which suggested megacolon. These dogs defecated in apparently normal fashion. No fecal impaction or diarrhea was encountered.

## 3 Hypogastric nerves

Four dogs were subjected to division of the hypogastric (sympathetic) trunks in the pelvis as control animals. Four centimeter segments

were excised from the hypogastric trunk on either side as in Figure 4, and damage to the nervus erigens was avoided

Colonmetrograms and barium enemas were carried out in these animals for periods ranging from 3 to 6 months. A total of 19 colonmetrograms were done. In 3 animals no change was observed in the filling curve of the colon. One dog showed hypotonic curves for 2 months after operation, but subsequent curves during the next 3 months were identical with the preoperative colonmetrogram. Barium enemas showed no change in the appearance of the colon in any of these animals. None of the animals developed any disturbance of bladder function or of defecation. No fecal impaction or diarrhea was encountered.

#### 4 *Observations in Patients (Vagus nerves)*

In 10 patients undergoing vagus nerve section for peptic ulcer colonmetrograms and barium enemas were carried out before operation and two weeks after vagotomy. In no instance did the colonic filling curve following vagotomy differ from the preoperative colonmetrogram. No changes were observed in the roentgenograms of the barium filled colon of any patient after vagotomy.

#### COMMENT

After division of the nervi erigentes in the dog colonmetrograms show consistently hypotonic curves. These flat curves indicate that section of the pelvic nerves results in reduction in the tone and reactivity to stretch stimuli of the colon of the anesthetized dog. These changes were not observed after division of the vagus nerves or the hypogastric nerves in dogs. No changes were observed in the colonmetrograms of clinical patients after vagotomy.

These observations are in accord with the experimental evidence as to the function of the motor nerves of the colon accumulated by Ivy and his associates (7) by electrical stimulation. Ivy found that electrical stimulation of the distal end of the divided pelvic nerves in dogs, monkeys, and pigs causes contraction of both longitudinal and circular muscular coats of the descending and distal colon. Stimulation of the vagus nerves, both in the neck and above the diaphragm was uniformly ineffective in producing a response of the colon in the dog, and only weak inconstant contractions of the cecum were occasionally observed in pigs and monkeys. Stimulation of the distal end of the



hypogastric nerves produced an inconstant circular contraction of the musculature of the distal colon in about half the animals studied.

The results of pelvic nerve division in this group of dogs are also in agreement with the colonometrographic observations made by White and his associates (6) on patients with destructive lesions involving the pelvic parasympathetic nerves, the cauda equina, and the sacral cord. In such patients the curves were found to be consistently hypotonic in type. Conversely, in patients with lesions of the motor fibers in the brain or in the descending spinal tracts the colonic filling curves were hypertonic in type. White concluded that the peristaltic contractions of the colon are a form of "stretch-reflex" with afferent and efferent arcs mediated through the pelvic parasympathetic nerves to the sacral segments of the cord.

The steep rise in intracolonic pressure and the powerful peristaltic contractions which occur as the normal colon is filled to capacity are not observed after division of the *nervi erigentes*. The most likely explanation for these differences in colonic activity is that expulsive contraction of the distal colon is abolished or greatly reduced by interruption of the pelvic parasympathetic reflex arc.

Although slight dilatation of the distal colon was observed in dogs after pelvic nerve division, nothing suggesting megacolon was encountered. No colonic dilatation was observed after hypogastric nerve section or vagotomy.

On the basis of these experimental observations it would seem reasonable to conclude that surgical attempts to alleviate spasm and hypermotility of the colon by interruption of its motor nerves had best be directed toward section of the pelvic parasympathetic nerves. If it should prove to be anatomically feasible in man to divide the pelvic parasympathetic fibers to the colon without interrupting the bladder fibers, an operative procedure to reduce the tone and reactivity of the distal colon might be developed which would be of benefit in certain instances of "spastic colitis" and possibly in early ulcerative colitis.

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## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

*A Primer of Electrocardiography* By BURCH AND WINSOR Second Edition 245 pp \$4 50  
Lea and Febiger, 1949

This book presents in logical order the fundamentals of electrical physiology which are necessary for the proper understanding of clinical electrocardiography. It is a book to be contrasted with the usual type of electrocardiographic manual which merely presents a series of clinical examples with brief explanations and interpretations. This primer is not just a collection of electrocardiographic patterns, but is a beautifully organized and clearly illustrated logical approach to the method and the meaning of the results in physiological terms. It is a superb text for students in a course of electrocardiography or for a practicing physician who wishes to understand something more than the usual empirical patterns obtained in cardiac disease.

It is obviously the result of much experience in the successful teaching and study of electrocardiography by these authors.

E V N

*Clinical Auscultation of the Heart* By LEVINE AND HARVEY 327 pp \$6 50 W B Saunders Company

This book follows as a natural sequence the already well known publication on clinical heart disease by the senior author of this present book. "Clinical Auscultation of the Heart" has been written to emphasize the importance of adequate physical examination in cardiological diagnosis, in which the value of auscultation and the information to be obtained by the use of the stethoscope is stressed. Chapters deal with the variations to be found in normal heart sounds, auscultatory findings in cardiac irregularities and the significance of cardiac murmurs. The text is amply illustrated with phonocardiograms recorded with simultaneous electrocardiograms and the book makes enjoyable reading. It is unnecessary to emphasize the fundamental clinical importance of much that is written, representing as it does the teaching and experience of one of this country's eminent clinical cardiologists. The therapeutic references in the book will be of considerable help to the practicing internist and the clinical approach to auscultatory problems at the bedside should be of help to future generations of students.

Realizing that this book has been written as a practical guide to clinical auscultation it seems a pity that the authors did not enlarge the scope of the book as the phonocardiographic tracings have been used mainly to illustrate and to help visualize points taken up in the discussion in the text. Phonocardiography has contributed much to a better understanding of the mechanism of heart sound production and with the large number of tracings included, greater use could have been made of them. In many instances it is impossible to determine accurately the duration and type of component vibration which has been recorded.

It would have been of great value if the authors had extended their discussion to a more complete analysis of the mechanism of heart sound recordings and also to debate

more fully some of the controversial aspects of heart sound production. However, the important emphasis made in this book is that often an all-important and life-saving diagnosis can be made by careful auscultation as long as the attending physician is aware of all the information which can be derived from such a relatively simple procedure. This book goes a long way in condensing this information and presenting it in an easily accessible form. As such it is recommended to all who confront this problem and to students who will always worry about the opening snap of the mitral valve.

B C S S

*Electrocardiography and Clinical Disorders of the Heart Beat* Sir THOMAS LEWIS 285 pp  
25/ Shaw and Sons Ltd, London

One need not comment at any length on this edition of these very popular sound treatises which should be read and re read by every student in medicine, whether he is in medical school or is a graduate who intends to continue to examine patients. It is fortunate that two works of this great physician have been combined in one compact, nicely printed volume.

E V N

*Handbook of Materia Medica, Toxicology, and Pharmacology* 4th Edition By FORREST R DAVISON 730 pp \$8 50 The C V Mosby Co, St Louis, 1949

The fourth edition of Davison's Handbook is divided into two parts. The first part considers principles of pharmacology, materia medica, prescription writing and toxicology, while the second contains a discussion of the individual drugs. The print is quite small. In general the book is an example of the categorical approach, which would make a distinct discipline of therapeutics, with little integration with current concepts of disease mechanisms. In addition, there are occasional major errors such as the confusion of rheumatic heart disease with bacterial endocarditis. Finally, there is a neglect of certain topics, as for example the discussion of electrolytes which is limited to six lines. For the above reasons the book cannot be recommended.

C G Z

*Measurements of Public Health* By F A E CREW pp xiii + 243 18 shillings Oliver and Boyd, Edinburgh, 1948

The author, who has had a distinguished career in the field of animal genetics, has more recently undertaken the professorship of public health and social medicine in the University of Edinburgh. The book is more specialized in scope than the title would suggest. It consists of a series of essays based on the data in the Registrar General's 1945 report for Scotland and is essentially a discussion of the state of the public health in Scotland, written in non technical language for interested laymen as well as medical men. The principal topics dealt with are the population structure by age and sex, birth- and fertility-rates, illegitimacy rates, marriage- and divorce rates, age at marriage, and death-rates by principal causes of death, including stillbirths and infant and maternal mortality. The book also includes topics such as multiple births, sex-determination and mutation, which appear rather remote from its main purpose, but serve as an indication that the author has not entirely deserted his old love. Unfortunately these sections seem to the reviewer to be the weakest from an expository point of view, since free use is made of the current terminology in genetics, not always with a clarifying explanation.

It may not be too much of a distortion to regard Professor Crew as a relatively "pure" scientist who has entered a career where almost any recommendation that is made involves intricate sociological, economic, political, and psychological questions. In this situation, one possible attitude for the pure scientist has been described by another outstanding geneticist, R. A. Fisher:

"The investigator undoubtedly best preserves both his scientific reputation and his peace of mind, who refrains from any opinion respecting human affairs, and declines the responsibility for any practical action which might be based upon the facts he has brought to light. If such a one were to admit that mankind might, with advantage, make more use of scientific knowledge of all kinds, his practical policy would, apparently, go no further than to advocate a more general diffusion of this scientific knowledge, its application to mundane affairs being relegated to others (unspecified) who might make it their particular business."

Professor Crew does not adopt this attitude, but he has not lost his scientific detachment and is in no hurry to rush in with a host of proposals for an immediate cure of Scotland's ills. Perhaps he might have ventured more than he has, for most of his definite recommendations seem rather obvious, and on some important and difficult issues it is not clear what he would propose.

As is well known, Scotland is likely to face the problems of an aging population, with a net reproduction rate fluctuating around unity and a drain by emigration on some of her best reproductive material. Unsatisfactory aspects of her public health are the relatively high infant and maternal mortality rates and the high illegitimacy rate (1 in 12 births). Professor Crew makes the point that if Scotland wishes to multiply this must be done now, since in the future the problem of caring for the aged will be a sufficiently great burden to deter her from assuming the additional load caused by an increase in children. With regard to emigration, she can afford to export her surplus women of marriageable age, but not her young married couples. He quotes investigations on the causes of infant and maternal mortality which indicate that in both cases a substantial reduction in death rates can be expected from increased availability of good medical care. He makes a moving plea for measures that will remove the stigma of illegitimacy from the child and ensure as good natal care for the unmarried as for the married mother.

William G. Cochran

*Pathology of Tumors* By R. A. WILLIS, D. Sc., M. D., F. R. C. P. 992 pp., 500 illustrations \$10.00 *Butterworth and Co., Ltd., London, and The C. V. Mosby Co., St. Louis* 1948

This is an excellent and very useful book on the pathology of tumors,—indeed, in the opinion of the reviewer, it is one of the finest in the field, in any language. It is a book that has grown out of a broad experience with tumors and a genuine devotion to the subject.

Those who may be so inclined may cavil at the fact that not all tumors about which they may seek information are described in the book, but any such objection cannot detract from the value of this comprehensive, well illustrated work. Because of the enormous variety of tumors, no single author has ever adequately described them all. Indeed, it is hardly possible that that could be accomplished in any treatise short of an encyclopedic compilation prepared by a great many different students of tumors, and even then many of the types and variations of tumors would, with little doubt, be inadequately treated.

Professor Willis never hesitates to express his own opinion plainly about matters concerning which opinions may differ. He does this, however, not in a dogmatic manner, but

with a clear, concise statement of the reasons for his preference, and he reserves the right to alter his opinion if future experience warrants doing so

The book is a refreshing one, for it was obviously written with pleasure, and it therefore represents one of the all too rare modern medical books in which the personality of the author lives in the pages, and which one can read with pleasure as well as with profit

A R R

*Problems of Early Infancy—Transactions of the Second Conference, March 1948* Edited by MILTON J E SENN, M D Price \$1 00 Pages 120 Publication of The Josiah Macy Jr Foundation, New York, N Y

The first part of this volume contains twelve reports whose wide range of subject matter is exceeded only by that of the discussions following each group of talks The first eight of these reports deal largely with the psychological problems of pregnancy, their possible influence on fertility, the cause and complications of pregnancy, the development of the fetus and the delivery of the patient The use of relaxation methods in obstetrics practice is discussed by Dr Albert Vollmer, and emotion in pregnancy and labor as related to childbirth by S W Goodrich, M D Other presentations include the myelination of the central nervous system in relation to function by Margaret A Kennard This subject is still but poorly understood However, there is strong evidence that myelination, as it results from use, helps to bring about increasing efficiency of function A memorandum on emotional disorganization in this culture by James Clark Moloney, M D offers a plea for affectionate understanding of the emotional needs of infants and children as a guide toward their education, rather than training He points out that many mothers because of their own narcissistic needs and neurotic tendencies are incapable of mother love and that they are unconsciously aided and abetted by many physicians and nurses whose authoritative and sometimes irrational behaviour place a cold screen of mechanical devices between mother and child The last presentation in this group by Dr Preston A McLendon is entitled "Modern Nurseries—New Design" It contains a description of the nurseries of the George Washington University Hospital This is a timely paper, obstetricians, pediatricians and Public Health authorities are becoming more and more aware that most hospital nurseries are inadequate to provide either rational mother-child relationship or adequate control of infection The breaking up of large nursery groups into small units as has been done in the new nurseries offer a practical approach to both of these problems

This volume contains as a supplement reports of five talks concerned with the impact of World War II upon the emotional development of children in the occupied countries of Europe Four of these were presented by European pediatricians visiting the International Congress of Pediatrics in New York in 1947 These give first hand information to American pediatricians The fifth report in this group was made by Dr David Levy who travelled abroad extensively shortly after the end of the war This is a very worthwhile little volume presenting a variety of challenging ideas to Americans interested in child development

J B H

*The Premature Infant* JULIUS H HESS, M D, EVELYN C LUNDEEN, R N 381 Pages 101 illustrations Price \$6 00 J B Lippincott Co, Philadelphia, May 1949

This is the new, thoroughly up-to-date second edition of the well known book on premature infant care by Hess and Lundeen Here is described in detail the care given to pa-

tients in the premature infant stations in the Sarah Morris Hospital, Chicago. The book should be of great value to Public Health nurses, and nursery personnel as nursing technique, procedures and teaching methods, both for hospitals and homes use, are outlined in the greatest detail. The chapters devoted to the medical care of the premature infant are complete only rather superficially but because of the detailed information given about some practical aspects of the problem, for instance, dosage of chemotherapeutic agents, methods of administering transfusions, etc., they should be of considerable value to the practicing physicians. The chapter discussing the prognosis for premature infants, particularly as regards mental development represents an important contribution to our knowledge in this direction. The final chapter in which city-wide and state wide plans for the care of premature infants are discussed is of particular interest. The concise description of the plans operating or being developed in eleven states should prove of value to Public Health officers and others interested in the all important phases of community planning, the only real way in which to reduce mortality rates among premature infants.

J B H

## BOOKS RECEIVED

- Atlas of Roentgenographic Positions in two volumes* By VINITA MERRILL 708 pp \$30 00  
C V Mosby Co , St Louis, Missouri
- Essentials of Orthopaedics* By PHILIP WILES 486 pp \$10 00 *The Blakiston Co , Philadelphia, Pa*
- Hematology for Students and Practitioners* Revised 2nd Edition, By WILLIS M FOWLER  
535 pp \$8 50 *Paul B Hoeber, Inc , New York*
- The Neurosis of Man* By TRIGANT BURROW 428 pp \$7 50 *Harcourt, Brace and Co , New York*
- The Practice of Refraction* By SIR STEWART DUKE ELDER, 5th Edition 317 pp \$6 25  
C V Mosby Co , St Louis, Missouri
- The Science and Art of Joint Manipulation* 2nd Edition By JAMES MENNELL 215 pp  
*The Blakiston Co , Philadelphia, Pa*





# EVIDENCE FOR THE PRESENCE OF RIBONUCLEIC ACID IN THE CYTOPLASMIC BODIES THAT APPEAR IN THE HEPATIC AND ADRENAL EPITHELIAL CELLS OF MAN IN ACUTE INFECTION

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Not infrequently there appear in the epithelial cells of the liver and adrenal cortex of man strongly basophilic cytoplasmic bodies of undetermined nature and significance. They vary in size and shape, some having a globular form, some appearing as straight or curved rods, and some having an irregular contour, their size ranges from less than a micron to several micra in diameter or length. They may be present sparsely or in profusion, and may be scattered indiscriminately in the cytoplasm or arranged in rows or palisades that lie along the margins of the cells.

It is strange that these cytoplasmic bodies in the human liver have been so generally overlooked and neglected, considering the frequency of their occurrence in pathological material, and their often striking appearance in the routine hematoxylin-eosin stain of tissue fixed in the ordinary fixatives. They receive no mention at all in treatises, text-books or systems dealing with the pathology of the liver or adrenals. For some years we have been impressed by their relation to severe infection, and that impression was confirmed by a study carried out in this laboratory several years ago by Santee (2), who found that in a series of unselected cases in which the liver and adrenals were examined histologically, the presence of the cytoplasmic bodies was associated with acute infection in the great majority of cases. While we certainly do not intend to imply that infection is the sole condition under which these bodies occur, it seems nevertheless clear that some factor associated with infection provides a particularly favorable stimulus or condition for their appearance.

In the course of a study of the relation of certain cellular constituents to the functional and reproductive potentialities of the liver cells (1), it became desirable to examine more closely the nature of the cyto-

plasmic bodies that form the subject of the present paper. The purpose of this report is to present evidence for the presence of ribonucleic acid in these bodies.

The possibility that the bodies in question might contain ribonucleic acid was suggested in the first place by the fact that they are markedly basophilic and, as is now recognized, cytoplasmic basophilia is often associated with the presence of ribonucleic acid. In the second place, these bodies bear some resemblance to the basophilic, so-called "protein storage" bodies of Berg (3), which are present normally in the liver cells of various animal species when the diet contains an adequate amount of protein, and several investigators have presented evidence for the presence of ribonucleic acid in the latter bodies in the hepatic cells of the normal rat (4, 5, 6, 15), an observation that we have confirmed. In a study of the nature of the basophilic bodies that become prominent in the human liver under pathological conditions, and of the factors responsible for their development, the present examination of their possible nucleic acid content was carried out.

As long ago as 1913, van Herwerden (7), in a pioneering study, demonstrated that certain basophilic cytoplasmic structures could be removed from animal cells by immersing the sections in a solution of "nuclease" prepared from spleen, and offered the observation as evidence for the presence of nucleic acid in those structures. In 1920, Walter Jones (8) prepared from pancreas a heat-stable enzyme that depolymerized ribonucleic acid, but not desoxyribonucleic acid, and in 1938 Dubos and Thompson (11) obtained from pancreas and other tissues a heat-stable enzyme, doubtless identical with that of Jones, which depolymerized ribonucleic acid and did not affect any other substrate, including desoxyribonucleic acid, against which it was tested. The latter investigators termed the enzyme "ribonuclease." Various observations had indicated a relationship between cytoplasmic basophilia and the presence of ribonucleic acid, and Brachet (9), in 1940, found that the enzyme prepared by the method of Dubos and Thompson removed cytoplasmic basophilia from various cells, whereas the nuclear basophilia, associated with the presence of desoxyribonucleic acid, remained unaffected. This observation has been confirmed by subsequent investigators in studies of various types of cells. We therefore sought to determine whether the bodies under present

consideration are susceptible to the action of ribonuclease, as a test of their possible ribonucleic acid content

We have found, as might be expected, that the enzyme when insufficiently purified is unsuitable for the specific histochemical demonstration of ribonucleic acid, apparently because of contamination with other enzymes which affect cellular constituents other than ribonucleic acid. In the experiments reported here, therefore, crystalline ribonuclease (Worthington Biochemical Laboratory, Freehold, N. J.) was used throughout. While crystallization is, of course, no guaranty of chemical purity, this crystalline product is demonstrably free from sources of error that become obvious when a less purified preparation of the enzyme is used.

The procedure adopted was as follows. Material from autopsies on cases of severe infection, in which the liver and adrenals exhibited well-preserved basophilic cytoplasmic bodies in the routine hematoxylin-eosin sections was used for study. The tissues had been fixed in Helly's fluid and embedded in paraffin. Freshly cut sections were deparaffinized in the usual manner, the precipitate of mercury salt from the fixative was removed with iodine followed by sodium thiosulfate, and the sections were thoroughly washed in tap water followed by distilled water. Slides having a large well for the test fluids were prepared by affixing two parallel strips of glass, cut from a microscopic slide, to the surface of another slide, with balsam or Duco Household Cement, or by fusion in a glass-blower's flame. The glass strips were placed three or four centimeters apart, leaving a space between them adequate to accommodate the length of the section of tissue. To facilitate handling, the under surface of each slide so prepared was cemented to a second slide, one end of which projected about two centimeters beyond the corresponding end of the upper slide.

A solution of crystalline ribonuclease in veronal acetate buffer was made up freshly immediately before each experiment, in the proportion of 1 mg. of the enzyme to 1 cc. of buffer. The buffer was prepared by adding 51 parts of M/10 veronal to 49 parts of M/10 acetic acid. The pH of the buffer, as determined with a Beckman glass electrode pH meter, varied between 6.75 and 6.79.

In the case of each section to be subjected to the enzyme, the ribonuclease solution was placed in the well of a prepared slide, and

the slide bearing the deparaffinized, washed section was inverted and placed upon the glass strips that formed the ends of the well. All air bubbles were removed, and more enzyme solution was added with a capillary pipette until the entire well was filled and the tissue section completely bathed in the solution. In each case a control was prepared by exposing a tissue section from the same block to the veronal buffer alone in the same manner. The test and control preparations were placed in moist chambers in an incubator at 37° for two to five hours, after which the slides containing the tissue sections were washed thoroughly in running tap water, then washed for 5 minutes in each of three changes of distilled water and, finally, were stained with Giemsa, by which the cytoplasmic bodies in question, in their unaltered state, are stained a deep purple.

It was found that whereas the basophilic bodies were unaffected by contact with the buffer alone, they were no longer stainable or visible in the cells that were exposed to the solution of ribonuclease, indicating that the enzyme had depolymerized a ribonucleic acid component of the bodies, to which their basophilia is due. The desoxyribonucleic acid of the nuclear chromatin was unaffected by the ribonuclease, and the nuclei of the cells exposed to the enzyme retained their property of becoming brilliantly stained with Giemsa.

The property of trivalent lanthanum ions to combine with nucleic acids, forming compounds of very low solubility, was applied by Caspersson (10), in 1936, to render cellular nucleic acids in tissue sections insoluble, so that they would remain "precipitated" *in situ* when the tissue section was exposed to the action of digestive enzymes. By this method he was able to study more accurately the localization of nuclear nucleic acids. Opie and Lavin (15) subsequently used this method to render cytoplasmic ribonucleic acid insusceptible to the action of ribonuclease. We therefore examined the effect of lanthanum upon the cytoplasmic bodies under present consideration. Sections of liver and adrenal, the cells of which contained these bodies, were deparaffinized and placed in a 0.2 M solution of lanthanum chloride ( $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ ) for three hours at room temperature, after which they were washed for 30 minutes in running tap water, followed by two changes of distilled water. Control sections, from the same blocks, were treated in the same manner with 0.6 M sodium chloride, instead

of lanthanum chloride. The sections were then exposed to ribonuclease in the manner described above. Giemsa stains showed that whereas the ribonuclease completely removed the basophilic bodies from the control sections, the bodies in the lanthanum-treated sections were definitely protected against the action of the enzyme, staining somewhat less intensely with Giemsa than control sections not exposed to the enzyme, but staining with clarity. This is additional evidence for the presence of ribonucleic acid in the cytoplasmic bodies under consideration.

Since it has been shown by Dubos and MacLeod (12) that bacteria can be robbed of their Gram-positivity by exposure to ribonuclease, indicating that ribonucleic acid is an important component of the bacterial substance responsible for Gram-positivity, sections of liver and adrenal containing the cytoplasmic bodies were stained with the Gram stain. The bodies were not Gram positive. This negative result is, of course, no valid evidence against the presence of ribonucleic acid in the bodies, for it is clear that the Gram-positivity of bacteria depends not solely upon the presence of ribonucleic acid, but also upon certain other factors that are not understood at present, among them a physical factor, for the mere physical disruption of Gram-positive bacteria suffices to render the residue Gram-negative in spite of its content of ribonucleic acid, recalling the loss of acid-fastness by tubercle bacilli that are mechanically traumatized (16).

#### DISCUSSION

The functional significance of the basophilic, cytoplasmic bodies that are at times so strikingly apparent in the epithelial cells of the liver and adrenal cortex of man, and which were the object of the present study, is not yet understood. We have not encountered them in the cells of other glandular organs, even in cases in which they were brilliant and abundant in the liver and adrenals.

It seems clear, from our observation of these bodies in pathological material over a period of years, that severe acute infections provide especially favorable conditions for their appearance. Indeed, the association is so close that when these bodies are prominent in the liver cells we have become accustomed to suspect a concomitant acute infection, and have learned that there is a high degree of probability

that that surmise will be correct, though infection is certainly not essential for their appearance, and they may be inconspicuous, faint or absent in cases of severe infection. In some of the latter instances their absence is probably the result of post-mortem changes that lead to the disappearance of the bodies. They appear to be highly susceptible to autolysis, and every degree of lysis, from loss of capacity to stain well to complete disappearance, can be seen in cases in which postmortem autolysis is in progress. Their fading and disappearance after the death of the cell is also obvious in freshly dead cells in foci of necrosis in livers in which they are abundantly present and brilliantly stained in the neighboring non-necrotic cells. Since, in routine autopsy material, the adrenal cortical cells commonly show more advanced autolytic changes than the hepatic cells, the bodies are, correspondingly, seen less often and may be less brilliantly stained in the adrenals than in the liver. Furthermore, it is of interest that the number and size of the bodies in the adrenal cortical cells bear an inverse relation to the amount of cortical lipid. The bodies are ordinarily absent from cells that are heavily laden with lipid, even when they are present abundantly and brilliantly in the adjacent lipid-free cells of the same section.

The present studies provide evidence that ribonucleic acid is an important constituent of these cytoplasmic bodies. In the first place, the bodies are basophilic, and there is much accumulated evidence that cytoplasmic basophilia is commonly associated with the presence of ribonucleic acid, in the second place, ribonuclease, which depolymerizes ribonucleic acid, robs the bodies of their stainability by basic dyes, and in the third place, lanthanum salts precipitate ribonucleic acid, and treatment with lanthanum chloride protects these bodies against the action of ribonuclease.

Nothing can be stated with assurance at present regarding the functional significance of these bodies. While they bear some resemblance to the familiar "protein storage" bodies described by Berg (3) in the normal livers of lower animals, as was stated above, and although both types of bodies contain ribonucleic acid, it would be premature to conclude that they are identical chemically and functionally. In the consideration of this point it is necessary to recall that the "protein storage" bodies are, with regularity, prominent under normal conditions in the hepatic cells of the animal species in which they occur, if

the diet has been adequate in protein. The bodies with which we are concerned, on the contrary, are certainly not present, in the form described here, with the same regularity under normal conditions, if, indeed, they ever occur normally. We have examined with care sections of the liver and adrenals from twelve unselected cases in which death occurred, in otherwise essentially normal individuals, either suddenly or within several hours following the precipitating cause. In four of the cases death resulted from trauma (autopsy nos 14822, 17724, 18153, 18933), in four, from rupture of a congenital aneurysm of a cerebral artery (autopsy nos 15513, 17589, 18094, 19426), in two, from suicide (autopsy nos 19988, 21692), in one, from anesthesia during an operation for myoma of the uterus (autopsy no 18459), and in one case, a young man apparently in perfect health until he died suddenly, no cause of death was found at autopsy (autopsy no 16312). While in several of these cases there were in the liver cells, small aggregates of cytoplasm which, when the cells were stained rather heavily with the basic dye, exhibited a very faint basophilia, the basophilia was scarcely, if at all, more marked than that of the remainder of the cytoplasm, and far from the striking basophilia of the bodies under consideration. In none of these cases were cytoplasmic bodies of the type under consideration detectable. If these bodies occur at all under normal conditions in man, they must be extremely sparse, inconspicuous and of irregular occurrence. Under certain pathological conditions, however, they appear in profusion and with striking prominence. It would appear, therefore, that if they do exist in the normal liver and adrenal of man, they must exist for the most part either as minute forms that are virtually invisible in ordinary preparations, and which become greatly enlarged, with a marked increase in ribonucleic acid content, under pathological conditions, or else they must exist in some precursor and different form, with different staining properties. In being absent, or infrequent and of very irregular occurrence under normal conditions, they differ from the "protein storage" bodies of the liver cells of lower animals.

Another difference resides in the fact that these bodies, when they are present in the liver, are commonly found, and often in profusion in the cells of all zones of the adrenal cortex also, whereas the "protein storage" bodies in the livers of normal lower animals are not accompanied by the presence of similar bodies in the adrenal cortex.

In this connection, it is of particular interest that we have found



cytoplasmic bodies of precisely the same appearance, staining properties and susceptibility to ribonuclease, in the adrenal cortical cells in the absence of infection, in two cases of *reticular zone hyperplasia associated with the adrenogenital syndrome*. The basophilic bodies in these cases were confined to the hyperplastic reticular zone cells. In one case (Autopsy No. 16090), these cells had crowded out virtually all of the cells of the zona fasciculata and zona glomerulosa, and the patient died from cortical insufficiency. The bodies were abundant in the hyperplastic reticular zone cells. None were present in the liver. In the other case (Surg. Path. No. 49-838), the proliferation of the reticular zone cells had replaced the zona fasciculata, but had left the zona glomerulosa intact. The hyperplastic reticular zone cells contained many basophilic bodies, but none were present in the cells of the zona glomerulosa. The liver was not available for study in this case. The adrenal had been removed surgically in an attempt to ameliorate the condition of the patient.

While, therefore, it is possible that the "protein storage" bodies and those under present consideration may be functionally identical, it would seem proper, in view of the apparent differences, to study each independently until further information establishes their identity or non-identity. Even if they prove to be identical it is worth pointing out that, although the bodies found normally in the lower animals have been termed "protein storage" bodies because of the assumption that they represent simply a reserve, emergency store of body protein, and while their presence is demonstrably dependent upon the ingestion of protein, their actual functional significance is not really understood. In fact, there are good reasons for regarding it as more probable that they represent loci of enzyme activity or enzyme synthesis, rather than a mere reserve store of protein. A variety of enzymes have been found in Claude's (13) "large granule" cytoplasmic fraction of the rat's liver cells, and that fraction is also rich in ribose nucleoproteins. It is true that the large granule fraction, which should contain the "protein storage" bodies, also contains the mitochondria, and the precise localization of the enzymes in the different types of granules is not yet established. At all events, the fact that the so-called "protein storage" granules shrink and even disappear when protein is withheld from the diet is no proof that these granules are merely reserve stores of protein,

as has been assumed from this fact. The same effect of a protein-free diet could be expected if the granules represent loci of enzymes for in the absence of dietary protein, the daily reparative synthesis of the protein components of enzymes would be expected to be impaired, with a corresponding progressive diminution in the size of any particulate enzyme aggregate. Indeed, Miller (14) has presented evidence that during a seven day fast the catalase, alkaline phosphatase, xanthine dehydrogenase and cathepsin activity of rat liver decrease together with the loss of liver protein, and that on a low protein diet there occurred a decrease in the one hepatic enzyme studied (arginase).

Do the basophilic bodies which become prominent in the human liver and adrenal under pathological conditions represent a degenerative phenomenon, or an expression of an increased activity of some normal function of the cells? We believe that there are reasons for regarding the latter as the more probable. When they become prominent during infection, to what particular disturbance caused by the infection is their appearance a response? It is obvious that the isolated effect of each of a variety of disturbances that infections bring into being can be studied in human pathological material in cases in which those individual disturbances were produced by causes other than infection, and studies of this nature in relation to the bodies under consideration are at present in progress in our laboratory, as is also an experimental investigation of other aspects of the chemical nature of the bodies, the factors responsible for their development, and their possible relation to recognized normal cytoplasmic constituents, such as mitochondria.

### CONCLUSIONS

Under certain pathological conditions there appear in the cytoplasm of the epithelial cells of the human liver and adrenal cortex sharply outlined strongly basophilic bodies the existence of which has been generally overlooked. Severe acute infections appear to provide a particularly favorable stimulus for their development in both liver and adrenal.

These bodies become prominent also, in the adrenal in association with hyperactivity of the cortical reticular zone accompanied by the adrenogenital syndrome.

The full range of conditions under which they occur, or increase in prominence, has not yet been defined

Exposure to crystalline ribonuclease, an enzyme which depolymerizes ribonucleic acid, robs these bodies of their basophilia, and results in their disappearance in stained sections, indicating that they contain ribonucleic acid. This is further indicated by the fact that treatment with lanthanum chloride, which renders ribonucleic acid insoluble, protects the bodies against the effect of subsequent exposure to ribonuclease

The nature of the residual chemical components of these bodies, their functional significance, and the precise conditions responsible for their appearance, are problems for further investigation

We wish to acknowledge the helpful suggestions given us during the course of this work by Dr Norman Weissman

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## EXPLANATION OF ILLUSTRATIONS

(Photographs by Miss Joan Wheaton)

All sections were stained with Giemsa, all photographs were taken at the same magnification

FIG 1 Liver from case of lobar pneumonia with abscess formation (Autopsy No 21639) The section was treated with veronal buffer before staining The cells contain many basophilic bodies

FIG 2 Section of liver from same block as Fig 1, exposed to ribonuclease before staining The basophilic bodies have disappeared The few dots that remain are granules of hemosiderin

FIG 3 Adrenal from same case as Fig 1, treated with veronal buffer before staining The cells contain many basophilic bodies

FIG 4 Section of adrenal from same block as Fig 3, exposed to ribonuclease before staining The basophilic bodies have disappeared

FIG 5 Adrenal from case of widespread tuberculosis with perforation of ulcer of rectum followed by pyogenic peri-rectal abscess (Autopsy No 17013) The section was treated with veronal buffer before staining The basophilic bodies have a predominantly peripheral localization in the cells

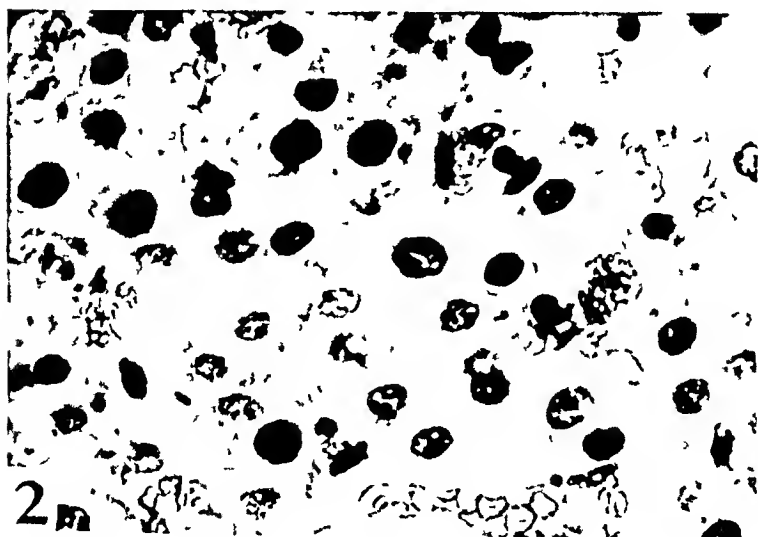
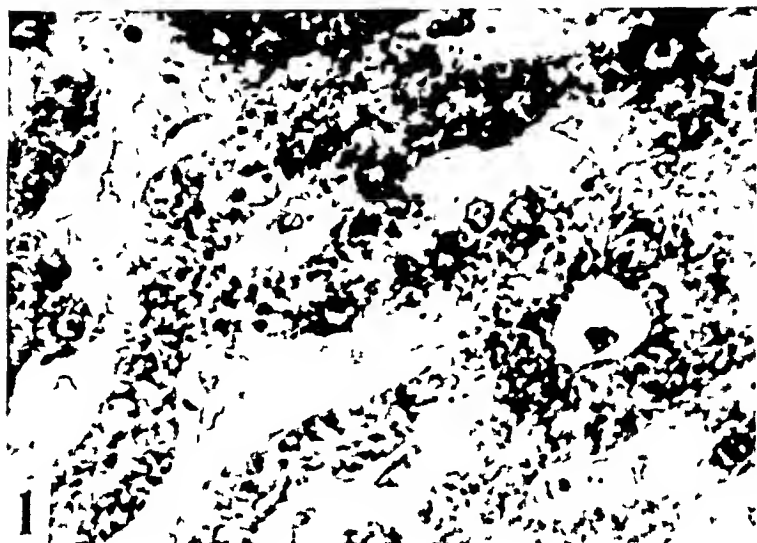
FIG 6 Section of adrenal from same block as Fig 5, exposed to ribonuclease before staining The basophilic bodies have disappeared

FIG 7 Section of adrenal from same block as Fig 3, treated with lanthanum chloride before exposure to ribonuclease The lanthanum salt has protected the basophilic bodies from the action of the enzyme

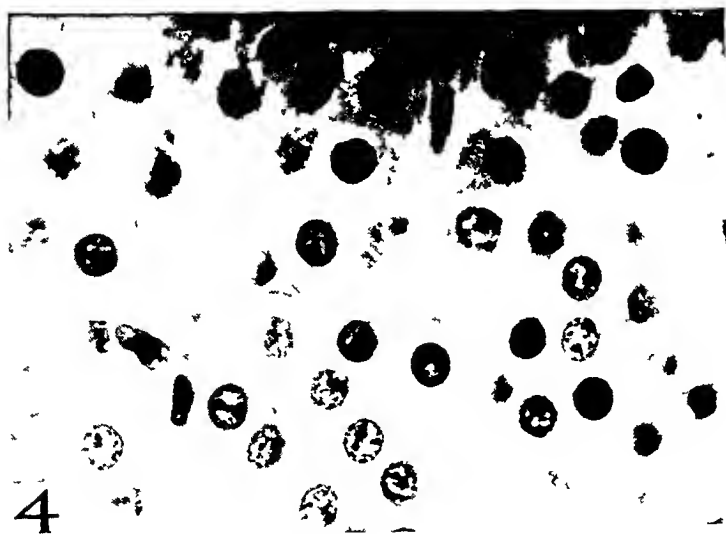
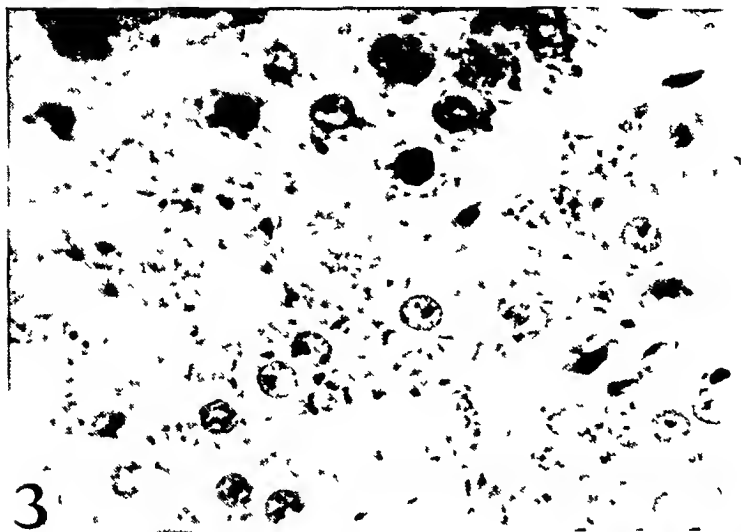
FIG 8 Section of adrenal from same block as Fig 7, treated with sodium chloride before exposure to ribonuclease The basophilic bodies have been destroyed by the enzyme Treatment with sodium chloride alone does not affect the bodies

FIG 9 Adrenal from case of reticular zone hyperplasia associated with precocious puberty (Surg Path No 48-838), treated with veronal buffer before staining There are many basophilic bodies in the hypertrophied reticular zone cells

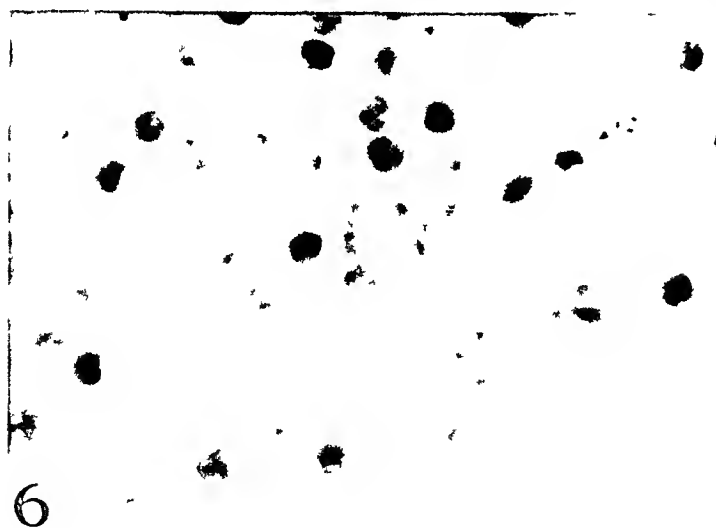
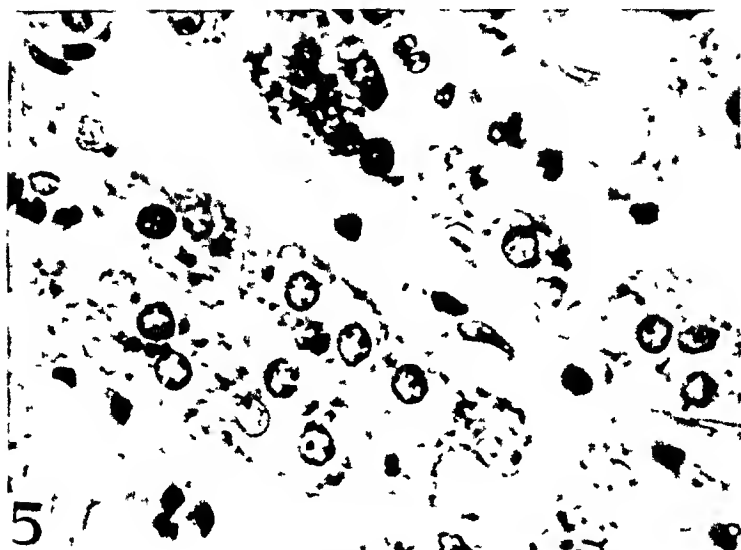
FIG 10 Section of adrenal from same block as Fig 9, treated with ribonuclease before staining The basophilic bodies have disappeared



FIGS 1 AND 2

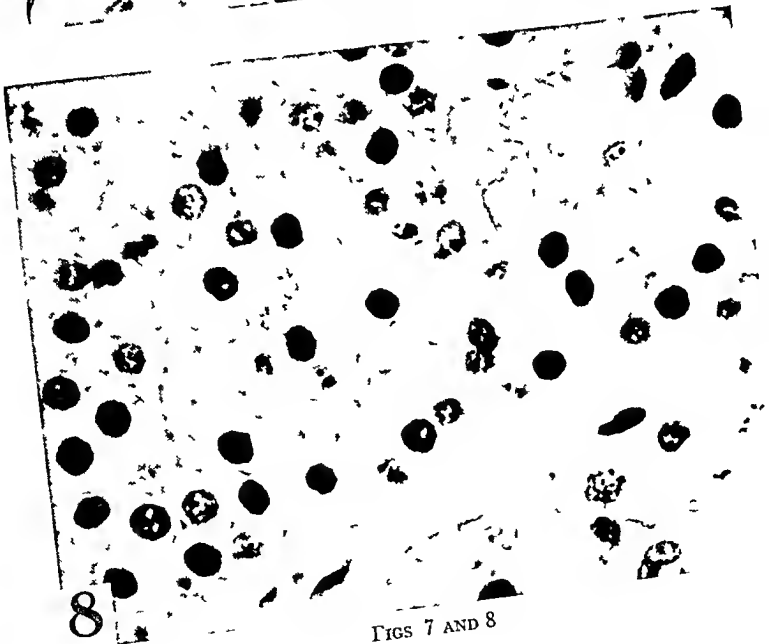
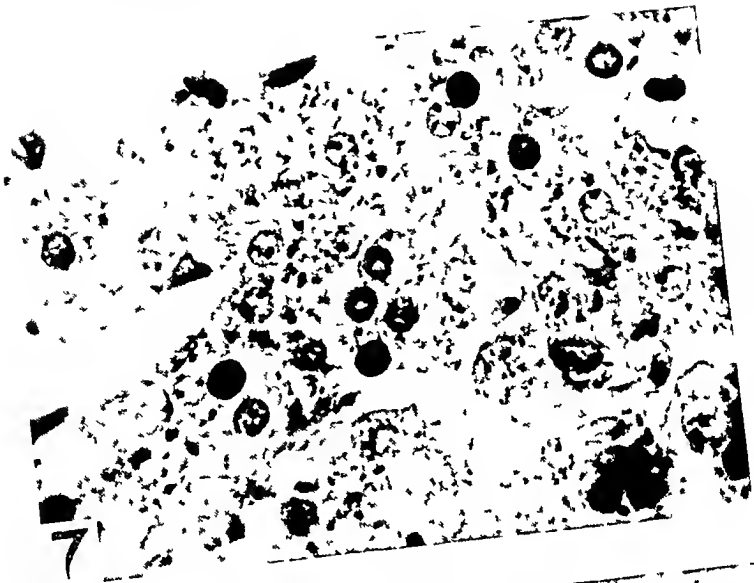


FIGS 3 AND 4

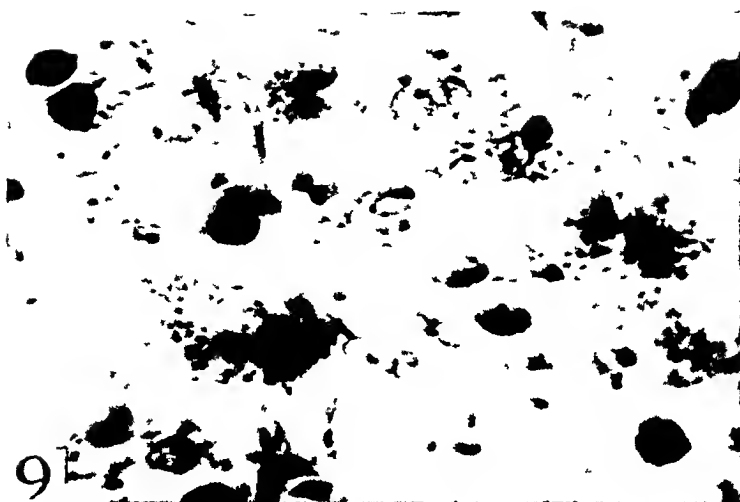


FIGS. 5 AND 6





FIGS 7 AND 8



FIGS 9 AND 10

# ELECTROCORTICOGRAPHY

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## INTRODUCTION

The recording of the electrical changes of the brain by electrodes placed directly upon the cerebral cortex has been termed electrocorticography.<sup>\*</sup> Although such techniques have been used in animals since the beginning of electroencephalography, in man the procedure has only recently been carried out extensively. Its use is much more limited than electroencephalography, since, when the brain is exposed, the lesion is usually apparent. Yet electrocorticography has a definite place in neurological surgery, being of great value in anatomical and physiological studies of the cortex, in the localization of subcortical tumors and in the determination of epileptogenous foci.

## TECHNIQUE OF ELECTROCORTICOGRAPHY

In early work (9, 14) the electrodes were merely laid on the cortex and not mounted on the skull. Surprisingly good records were obtained by this technique but the number of electrodes used was usually limited, only two being present on the cortex at once. For multiple recording some type of electrode holder, capable of being firmly clamped to the skull, is necessary. Several such types of assembly have been employed, perhaps the earliest being that used by Jasper (4). There are a number of models available at this time, all have similar principles but vary in design. The one used in this clinic (fig. 1) was devised by one of us (C. M.) and consists of a lucite plate in which 10-16 fine tubular electrodes are inserted, each upon a universal joint. The plate is attached to the skull by a clamp. Contact with the cortex is made by small

\* Electrocorticography is to be distinguished from recording of scalp potentials (electroencephalography) and from the recording from subcortical structures with needle electrodes.

silver balls mounted on very light springs at the ends of wires floating in the tubular probes so that the pressure upon the cortex is always light. In some electrodes the cortical contact consists of a wick which is kept moist (4, 14). The electrodes should be freely movable so that they may be placed at any point over a wide area of the cortex.

Although a completely shielded room is desirable, electrocortico-graphy may be carried out in the ordinary operating theatre with a good



FIG. 1 THE ELECTRODE HOLDER

electroencephalograph, provided a few precautions are observed. The patient and operating table should be grounded to the electroencephalograph. Electrosurgical units, electric motors and sometimes the electric lights of the room must be disconnected at the wall plug. With these special arrangements usually a good record from the cortex may be obtained free of artifact. If spikes are interfering in all channels an electric motor 30-50 feet away in an adjoining room may be at fault.

Occasionally such artifacts are in the line voltage and only with difficulty eliminated

#### NORMAL ELECTROCORTICOGRAM

The traces obtained from the exposed cerebral cortex differ from those obtained from the scalp in two main characteristics (11) First, the amplitude of the cortical activity is 3-4 times greater than when sampled through the calvarium and scalp. Second, rapid oscillations in electrical activity are not distorted in the electrocorticogram and hence appear as spikes, whereas in the electroencephalogram interference by activity from adjacent areas or distortion by the distributed capacity of the intervening tissues may blunt their peaks. The characteristic of the waves must be relied upon in the analysis of the electrocorticogram, for obviously, comparisons cannot be made with comparable areas of the opposite side of the head. Usually only a part of one or more lobes of the brain is exposed so that the actual sampling of cerebral activity is from a very limited field even when eight channels are used. Usually bipolar recording from the brain is found most practical (4, 7, 9, 10, 14, 15) for an "indifferent" point may not be in the surgical field or may introduce artifactual disturbances.

It has been said that the electrocorticogram is dependent upon the cytoarchitectural structure of the cortex and that different areas have typical frequencies and patterns (6). In electroencephalography, the occipital, posterior parietal and temporal regions have an alpha rhythm (8-11/sec) and the frontal regions a beta rhythm (14-22/sec). This is also true for electrocorticography in the absence of general anesthesia, but the frequencies and pattern often change (fig. 2), and except for a few generalities there cannot be said to be a specific pattern for any one area (9, 10). Although at times it may be possible to recognize an area by its rhythm, as a rule, under controlled conditions, such prediction is no better than chance.

Anesthesia plays a great rôle in the characteristics of the electrical activity (fig. 3). Our experience has been confined to three states of anesthesia, local infiltration of the scalp, general ether anesthesia and intravenous pentothal anesthesia. Ether anesthesia causes a marked slowing of the cortical frequencies (9) with some increase in amplitude (fig. 3 C). This is present in all regions of the brain. Such slow activity

obscures all normal alpha rhythm and seems to depress the general excitability of the cerebral cortex so that epileptic discharges are suppressed and rarely seen. Pentothal anesthesia, at least in the surgical planes, augments the frequency and amplitude from all cortical regions.

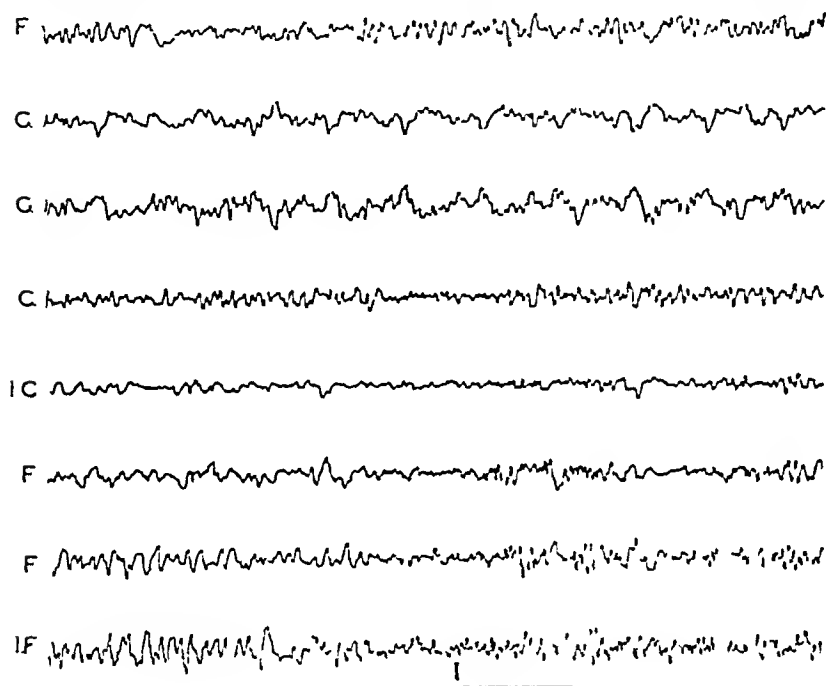


FIG. 2. Electrocorticogram of a patient operated upon under procaine infiltration of the scalp to show the marked changes in the spontaneous cortical activity. Note the transition from slow irregular activity in the frontal regions to regular 20 per second waves. The abbreviations are as follows: C, precentral cortex; I, frontal (prefrontal) cortex; IF, inferior frontal cortex. The horizontal line at the base of the record indicates an interval of one second, the vertical line a calibration of 200 microvolts.

(fig. 3 B). It does not seem to depress the cortical activity unduly, for epileptic discharges may still be seen. In general, the most accurate results can be obtained from electrocorticography when the craniotomy is done under local anesthesia, but at times satisfactory results may be obtained under general anesthesia. In epilepsy, when the subjective

responses of the patient are important, the bone flap may be reflected at a preliminary operation under general anesthesia and the cortical exploration carried out a week or ten days later with the scalp anesthetized with procaine

Morphine, in the doses usually employed to allay anxiety, does not seem to depress cortical activity

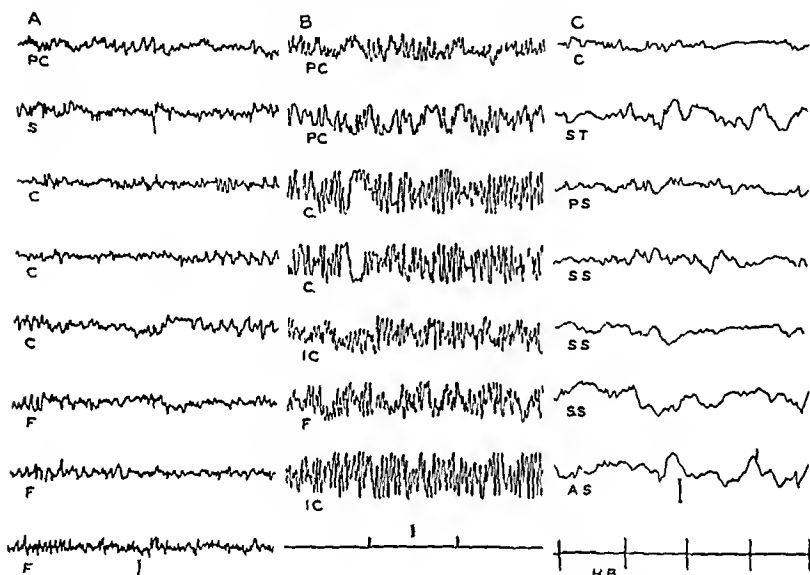


FIG 3 Electrocorticograms to show the effect of anesthesia on the trace A Procaine used for anesthesia of scalp B Pentothal sodium intravenously C Ether The abbreviations used are as follows A S, anterior Sylvian cortex, C, precentral cortex, F, frontal (prefrontal) cortex, H B, heart beat, I C, inferior cortex, P C, postcentral cortex, P S, posterior Sylvian cortex, S, Sylvian cortex, S S, superior margin of Sylvian fissure, S T, superior temporal gyrus

The vertical lines at the base of the traces indicate a calibration of 200 micro volts, the horizontal line at the bottom of the second trace indicates intervals of one second

Electrocorticography is subject to two influences not encountered in electroencephalography, which may produce artifacts in the traces—namely, brain pulsations due to respiration and cardiac action. On the other hand, muscle artifacts rarely are encountered in electrocorticography. The spiky waves produced by vascular pulsations may be recognized by their regularity and time relationships to an EKG put





The study of fiber pathways, using strychnine neuronography has not proved very valuable in man. The strychnine spiking is rarely well developed in man and usually may be obtained only from a small area about the site of the application of the drug. Rarely does it give rise to spiking in areas farther than a few millimeters from this locus. A number of other analeptic drugs have been placed upon the human cortex with similar disappointing results. Metrazol, which fires the macaque cortex for 15 to 20 minutes is practically ineffective in evoking spike potentials in the human cortex unless an epileptic focus is present. Picrotoxin, if injected intracortically, may induce spike potentials but the technique has not been used to any great extent. It would seem that the present methods of neuronography in man are unsatisfactory. Perhaps some modifications will give more fruitful results.

Physiological observations of human cortical function are being made at the present time by an analysis of the cortical responses along the lines that Bishop and others have used in lower animals. The subcortical spontaneous epileptic spike may be used to study the cortical response in man (3). Other physiological studies have confirmed for man the applicability of phenomena previously shown in lower animals such as cortical extinction following a convulsive seizure.

#### LOCALIZATION OF TUMORS

Foerster and Altenburger (2) in 1935 showed that a tumor exposed at operation has no electrical activity but that compressed or infiltrated cortex at the margin of the neoplasm has abnormal activity characterized by slow waves. The preoperative localization of brain tumors on the basis of such delta activity is of great value to the neurological surgeon, but once a bone flap has been made the tumor is usually visible. Obviously electrocorticography is of little value in such lesions, but at times it may aid in the localization of a subcortical lesion (14, 15, 16). Slow waves interfering with normal frequencies usually indicate an underlying lesion (Fig. 5). Such slow waves may have a frequency of a half to two per second and an amplitude of 100 to 200 microvolts. A series arrangement of bipolar recording electrodes will enable a precise localization by determination of out of phase activity. This technique is applicable both to tumors of the cerebral hemispheres and cerebellum (Fig. 5A and 5B). Our limited experience with electrocortico-



electrical stimulation, (3) production of a long lasting after-discharge or (4) the initiation of spiking by local or systemic administration of metrazol

*Focal spontaneous spiking* In epileptics, the spontaneous electrical activity of the cerebral cortex has no constant pattern. The predom-

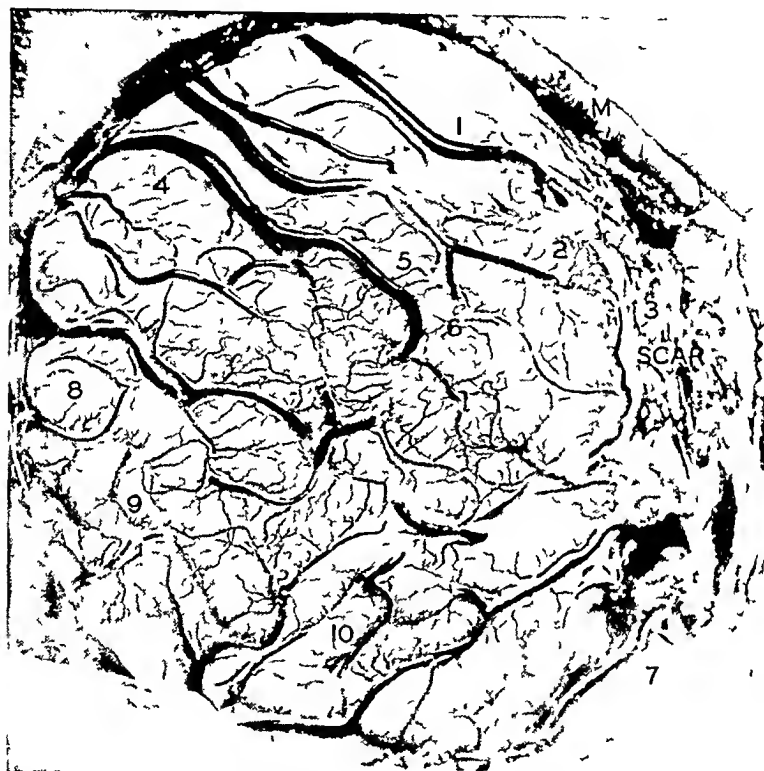


FIG 6 Photograph of the exposed brain of a patient suffering from posttraumatic epilepsy. The scar and initial electrode setting are shown.

inant frequency varies from approximately 1 to 26 per second, but usually falling in the 8 to 11 per second range. About a scar, slow wave foci are consistently found (8) but are considered to be indicative of brain damage and not of epileptic activity. From the cortex adjacent to a scar, focal spontaneous electrical alterations, such as isolated spikes, bursts of spikes or spiky waves are present in approximately

one half of the posttraumatic epileptics examined. These abnormalities are usually confined to one gyrus and are present in an area of only a few square centimeters. Such spontaneous abnormalities are rarely associated with any clinical phenomena either subjective or objective.

The spike activity takes the form of individual monophasic or more often diphasic spikes, bursts of spikes or runs of spiky waves. This con-

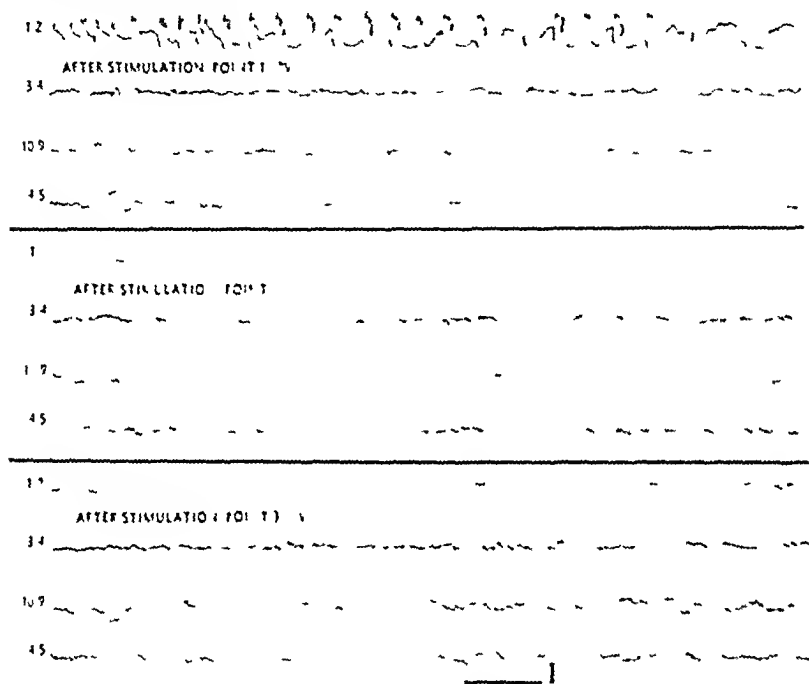


FIG. 7. Lithotrocorticrograms following stimulation of points 1 to 3 as indicated. The remainder of the points stimulated sequentially at 1 minute intervals give no prolonged after discharge.

tinuous activity is usually confined to a relatively small area of the cortex and may change from moment to moment. Yet it was found in one patient to be present for many minutes.

*Induction of the aura by electrical stimulation of the exposed cortex.* The hyperirritable characteristic of the focus is confirmed by electrical stimulation. Ordinarily, representative points of the exposed cerebral cortex are stimulated by a sine wave current of 1.5 to 2.5 volts for 5

seconds. The electrical activity of the cortex and the motor and sensory responses are recorded. If no change is noted in the electroencephalogram after one minute another point is stimulated and so on until the entire cortex is mapped. The motor cortex can usually be located in this manner without provoking a convulsive seizure. A more accurate mapping of the motor responses may then be carried out. Stimulation



FIG. 8. The electrodes were grouped about the suspected epileptic areas shown.

of the spiking area usually induces the patient's aura with or without a clinical or electrocorticographic seizure.

*Production of a long lasting after-discharge.* Occasionally an after-discharge occurs for a few seconds at the point of stimulation. Such a short lasting response up to 10 seconds is considered physiological. However, when the area spontaneously firing is stimulated with a cur-

rent too weak to elicit a response from normal cortex, a long lasting after-discharge usually occurs. If spontaneous spiking is not present,

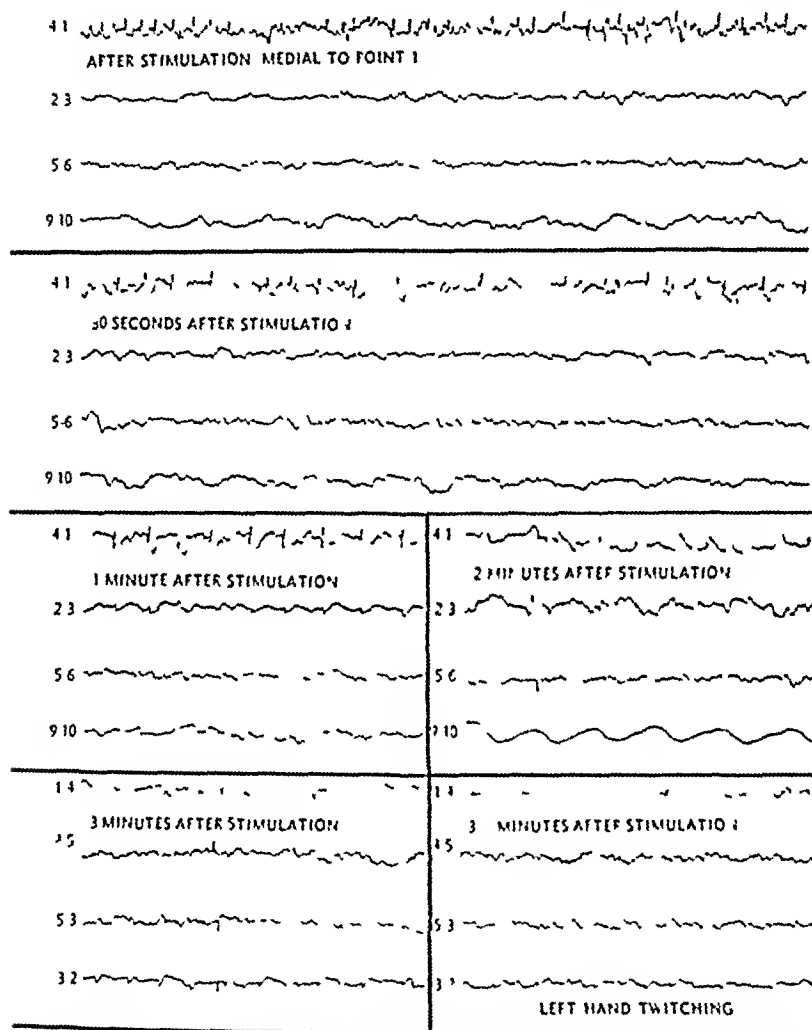


FIG 9 Electroencephalograms at intervals after stimulation of point 1 showing the focal after-discharge

generally an area of cortex, adjacent to the scar in posttraumatic epilepsy can be found which, when stimulated, causes a long lasting

spiky discharge. In one case this epileptic discharge lasted as long as 28 minutes. During this time the cortical activity of surrounding areas of the brain remained normal. During the period of focal epileptic discharge the patient thought he was quite normal and to all outward appearances seemed his usual self. He responded to simple questions and carried out simple commands and arithmetical problems. Only if the stimulation and after-discharge are in the motor areas or associated with an aura does consciousness appear to be impaired.

With the after-discharge there may or may not be the sensory or motor phenomena such as characterized the aura of the patient's attack. Of 33 cases, so activated, 13 had no clinical phenomenon with

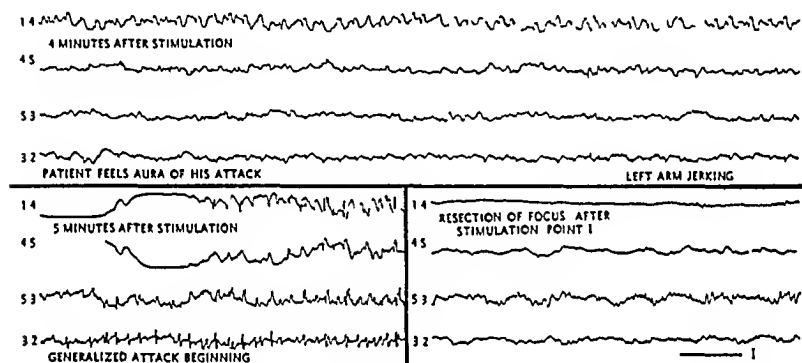


FIG. 10. The after-discharge spreads as the patient feels the aura and the seizure begins. The last trace shows the absence of after-discharge following the cortical resection.

the after-discharge. The remainder had sensory or motor focal manifestations which progressed in seven cases to a generalized seizure. The majority of the 13 cases having no motor or sensory phenomena associated with the after-discharge had no aura with their spontaneous attacks.

The after-discharge shows a tendency to build up during the first ten seconds and then assumes a fairly constant pattern. The frequency of the spiky waves decrease as the amplitude increases during the buildup. This is also true of the ending of the after-discharge. The pattern after the initial state may assume almost any form, but usually the hypersynchrony can be classified into one of five types: (1) spikes,

(2) saw tooth phenomena, (3) spike and dome forms, (4) phasic waves and (5) dicrotic waves and spikes. These forms may be continuous or paroxysmal, and/or regular or random.

*Initiation of spiking by local or systemic administration of metrazol*  
In the early explorations, when spontaneous cortical spiking was not evident, small amounts of metrazol were injected intravenously to

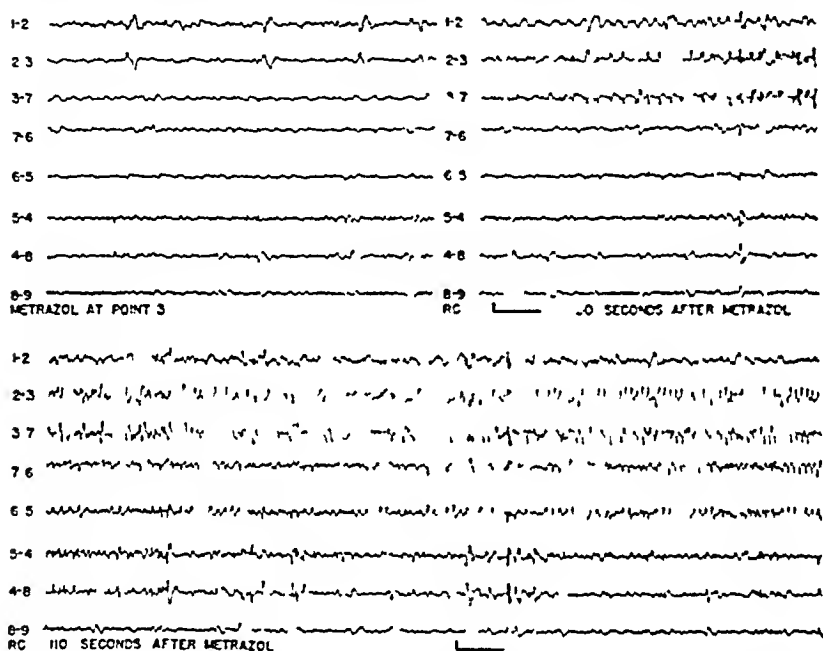


FIG. 11. Electrocorticograms showing the activating effect of a small amount of metrazol on a cotton pledget placed on an epileptogenic focus. In spite of the marked spiky activity the patient had no symptoms or signs of neurological dysfunction.

initiate the abnormal cortical activity. Our fear of the induction of a generalized seizure led us to abandon this technique. We discovered later that the foci in experimental epilepsy are hypersensitive to the local application of metrazol. In man we find that the intracortical injection of 0.1 cc of the usual metrazol solution produces no electrocorticographic changes in normal brains. But in epileptic foci even this small amount will initiate self-propagated discharges. In some cases



this technique gives highly satisfactory results but in other cases the areas adjacent to the real epileptic focus appear to be as hypersensitive to metrazol as the focus so that a clear-cut localization is not obtained

#### SUMMARY

Electrocorticography may be carried out in the ordinary operating room if certain precautions are taken

Anatomical and physiological studies of the cerebrum are quite feasible in man. The sensory representation has been studied by evoked potentials. Many characteristics of cortical physiology, especially associated with convulsions, may be explored by electrocorticography in man.

Subcortical brain tumors may be localized by the presence of slow wave activity over the tumor.

Electrocorticography is particularly valuable in the localization of epileptic foci. Cortical exploration should be carried out under local anesthesia. The epileptogenic focus may be determined by (1) focal spontaneous spiking (2) induction of a convulsive aura by the electrical stimulation of the cortex (3) production of a long lasting after-discharge and (4) by the initiation of spiking by local or systemic administration of metrazol.

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# STUDIES ON THE CHEMICAL DIFFERENTIATION OF DEVELOPING CARTILAGE AND BONE

## I GENERAL METHOD ALKALINE PHOSPHATASE ACTIVITY\*

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In recent years much information has been accumulated on chemical changes which accompany growth and differentiation of the organism and its constituent tissues. To carry out such studies it is necessary to employ animals at various stages of development in order that progressive periods of morphological differentiation can be correlated with quantitative chemical determinations. Developing cartilage and bone, however, are unique among tissues in that all stages of their development may be found in a single specimen whether it be removed at an appropriate time in utero, at birth, or thereafter during the stage of active growth. Such tissues are therefore eminently suited for correlating the morphological alterations with the chemical changes which take place during growth and differentiation. In certain instances, of course, qualitative histochemical reactions may be employed and are useful (1). Such techniques are sometimes open to criticism, however, we have therefore turned to more quantitative techniques.

The present paper, which is the first of a series on the biochemistry of developing cartilage and bone, will describe the general method and its application to the study of one enzyme, alkaline phosphatase.

### GENERAL METHOD

Two requirements must be met before utilizing the method to be described below. First, the specimen must be large enough to furnish sufficient material for chemical analysis. Second, the entire line of ossification must be in as level a plane as possible. In the specimens of cartilage and bone which have been studied the ribs from man and dog fulfill these two criteria. Long bones cannot be used since the diaphyseal portion of the cartilage is concave.

\* Aided by a grant from Mead Johnson and Company

The method consists in making horizontal slices of varying thickness through the cartilage in a plane parallel to the costochondral junction. One half of each slice is used for chemical study the opposite half is prepared for histological examination.

Slices are cut on a Spencer Automatic Clinical Microtome (No 888) whose knife clamp has been removed and replaced by a horizontal plate to which is fixed a razor blade (Durham-Duplex) (Fig 1). An object clamp (Spencer No 885) replaces the carbon dioxide freezing

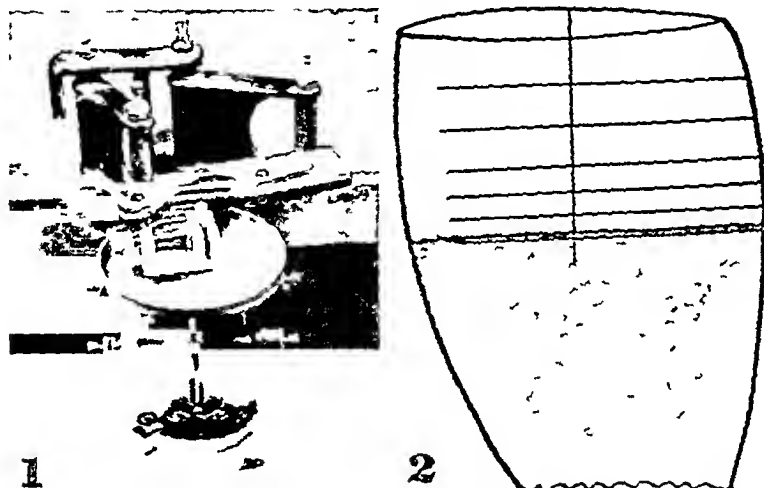


FIG 1 Microtome assembly showing plate which is used to hold the razor blade in an horizontal plane

FIG 2 Schematic representation of slicing technique. Horizontal cuts are made three quarters or more through the cartilage. The final vertical slice frees the segments on the right for chemical analysis while those on the left are held together in one piece by the un-sliced cartilage at the extreme left.

stage. The rib is clamped by the shaft in a vertical position with the line of ossification in as horizontal a plane as possible. This may be facilitated by transillumination of the region. The cartilage is cleaned of any adhering muscle or fat. The razor blade is then brought level with the line of ossification and depending on the desired thickness the stage is lowered by the vertical feed screw for a distance corresponding to the thickness of and number of slices desired. While the cartilage is steadied by manual counterpressure the blade is brought completely across the cartilage. The vertical feed screw is then turned and the

required number of slices of known thickness are made three quarters through the cartilage and on down to and including the cartilage shaft junction. When these transverse cuts have been completed, a mid-longitudinal slice is made from the free end of the cartilage down into the shaft (Fig. 2). This frees each slice nearest the blade, the fellow

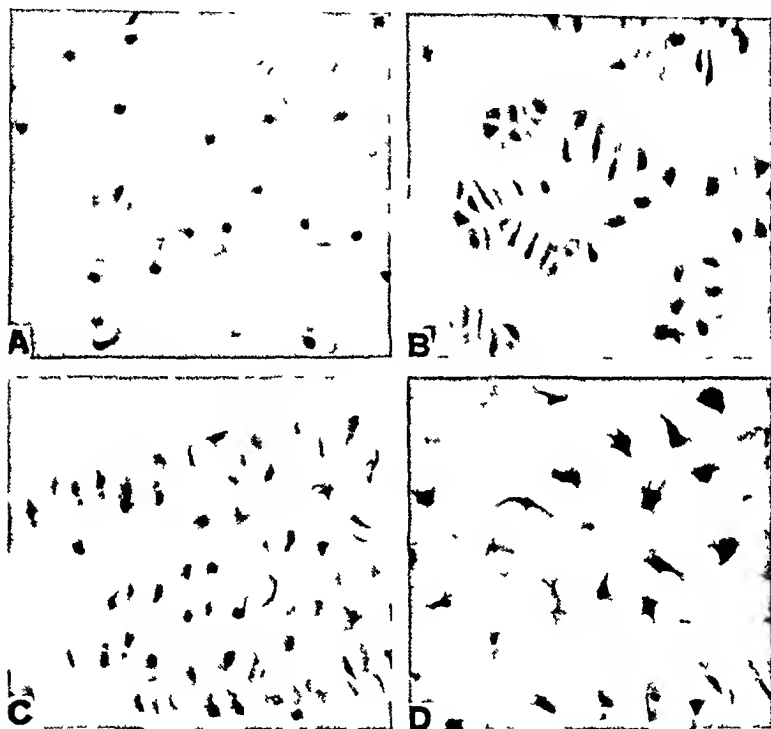


FIG. 3 Cartilage cells from various regions of differentiation, A, most undifferentiated, D, hypertrophic, most fully differentiated cells. See text for further explanation.

of each of these is held to the others by the remaining quarter which has not been sliced. The free halves are placed serially in a Petri dish and weighed on a torsion balance either immediately or after drying at  $105^{\circ}\text{C}$ . The entire hemisection of partially sliced cartilage is fixed in any desired solution and prepared for histological examination in the usual way, thus providing a series of segments separated by the incisions made by the razor blade.

Regions of the cartilage have been graded A, B, C and D depending on their degree of maturation. The cells in each of these 4 regions are shown in Figure 3. Region A is composed of the smallest, most undifferentiated cells. In region B the cells have become larger and are found in pairs, the cells in this region are undergoing division. In region C the cells are larger and have arranged themselves into rows. Region D consists of hypertrophic cells just above the invading blood vessels. In the present paper and those which will follow the slices which are studied will not necessarily consist entirely of each of these regions. There naturally is overlapping and the grading which is given is an average.

#### METHOD FOR PHOSPHATASE ACTIVITY

Slices are placed immediately without weighing in small serological tubes containing 5% beta glycerol phosphate solution buffered with sodium barbital at pH 10.9. They are shaken for 2 hours in a water bath maintained at 37°C. After the slices have been removed, 10% trichloroacetic acid is added and the phosphorus content of an aliquot determined by the method of Iske and Subbarow (2). The slices are dried at 100°C and weighed on a torsion balance. The alkaline phosphatase activity is expressed as milligrams phosphorus liberated in an hour per gram of dried tissue.

In the determination of periosteal phosphatase activity the pleura is removed from the inner surface of the rib and small segments of periosteum are stripped from the underlying cortex. These are treated in the same way as are the cartilage slices described above.

For the histological demonstration of alkaline phosphatase activity the technique of Gomori (3) has been applied to undecalcified sections, using 2 preparations: one incubated with glycerol phosphate and both then stained with silver nitrate.

#### RESULTS

In Table I is shown phosphatase activity at varying levels of differentiation (rated A to D) in the costal cartilage of the dog. There is slight yet definite activity in the zone of undifferentiated cells, as the region of hypertrophic cells is reached, a sudden increase in enzyme activity occurs. Quantitative values of periosteal phosphatase activity are likewise shown in Table I.

TABLE I

*Correlation Between Cartilage Cell Differentiation and P ase Activity\* in the Dog*

NUM BER	P ASE ACTIVITY* VERSUS DIFFERENTIATION OF CARTILAGE CELLS						P ASE ACTIV- ITY IN PERIO- STEUM
	Slice 1	2	3	4	5	6	
1	1 1(A)	2 7(A)	3 8(A,B)	8 0(B,C)	20 5(C,D)	33 7(D,S†)	9 4
2	5(A)	5(A)	5(A)	2 0(A,B)	7 8(C)	21 3(D)	6 1
3	5(A)	5(A)	5(A)	8(A)	2 6(B,C)	13 0(C,D)	6 5
4	8(A)	8(A)	8(A)	9(A)	2 5(B,C)	6 4(C,D)	4 0
5	2(A)	2(A)	7(A)	1 5(B)	3 0(B,C)	3 8(C)	2 9
6	2(A)	2(A)	4(A)	7(B)	3 6(C)	8 1(D)	4 8
7	2(A)	2(A)	2(A)	3(A)	1 8(B,C)	3 9(C,D)	2 0
8	3(A)	3(A)	5(A)	5(A,B)	3 0(B,C)	8 7(D)	3 2
9	2(A)	3(A)	3(A)	4(A)	4 0(B,C)	4 2(C,D)	2 0

\* Expressed as Mg P liberated by 1 gram dry cartilage or periosteum per hour

† S—Shaft of rib

TABLE II

*Correlation Between Cartilage Cell Differentiation and P ase Activity\* in Children*

NUMBER	AGE	DIAGNOSIS	P ASE ACTIVITY * VERSUS DIFFERENTIATION OF CARTILAGE CELLS						P ASE ACTIV- ITY IN PERIO- STEUM
			Slice						
			1	2	3	4	5	6	
669	3 mo	Bronchiectasis	1 0(A)	1 0(A)	1 0(A)	2 0(B)	12 6(B C)	24 7(C D)	10 2
648	3 mo	Pneumonia	1 1(A)	1 2(A)	1 4(A)	3 0(B)	10 0(C)	14 6(D)	7 1
653	3 mo	Cardiac hyper trophy	4(A)	6(A)	9(A)	5 1(B)	6 7(C)	8 8(D)	3 2
649	6 mo	Cong Mal Heart	5(A)	5(A)	5(A)	1 9(B)	7 4(C)	10 1(D)	5 3
702	6 mo	Obstruction Trachea	5(A)	5(A)	5(A)	1 5(A B)	3 1(B C)	9 6(C D)	9 4
712	9 mo	Hydrocephalus	6(A)	8(A)	6(A)	8(A B)	2 9(B C)	4 9(C D)	2 3
727	15 mo	Cong Mal Heart	4(A)	4(A)	4(A)	4(A)	6(B)	11 5(C D)	7 8
765	2 yr	Cong Mal Heart	3(A)	3(A)	4(A)	4(A)	1 7(B)	1 0(C D)	2 8
671	3 yr	Cong Mal Heart	4(A)	5(A)	8(A)	7(A)	1 5(A B)	9 2(C D)	5 6
674	3 yr	Hydrocephalus	1 3(A)	1 4(A)	1 3(A)	2 1(B)	2 4(B C)	18 0(D S†)	—
725	3 yr	Neuroblastoma	6(A)	6(A)	6(A)	6(A)	2 3(B C)	4 7(C D)	4 8
748	3 yr	Cong Mal Heart	2(A)	2(A)	3(A)	3(A)	6(B)	4 9(C D)	5 1
719	4 yr	Meningitis	4(A)	4(A)	4(A)	4(A)	1 4(B C)	6 3(D)	3 0
763	6 yr	Cong Mal Heart	3(A)	3(A)	3(A)	3(A)	3(A B)	1 1(C)	3 0
718	7 yr	Polomyelitis	4(A)	4(A)	4(A)	4(A)	3 5(B C)	4 8(C D)	2 4
755	7 yr	Cong Mal Heart	4(A)	5(A)	5(A)	1 5(B C)	1 9(C D)	—	1 9
665	12 yr	Hydrocephalus	1(A)	2(A)	1 2(B)	1 5(B C)	2 3(C D)	—	2 0
704	14 yr	Rheumatic Ht Dis	4(A)	4(A)	4(A)	1 7(B)	5 6(C D)	4 3(D S†)	8 6

\* Expressed as Mg P liberated by 1 gram dry cartilage or periosteum per hour

† S—Shaft of rib

In Table II are summarized values of phosphatase activity at varying levels of cartilage cell differentiation and in the periosteum of a group of children dying of a variety of causes. These data indicate the variability in quantitative results which are encountered. Age as well

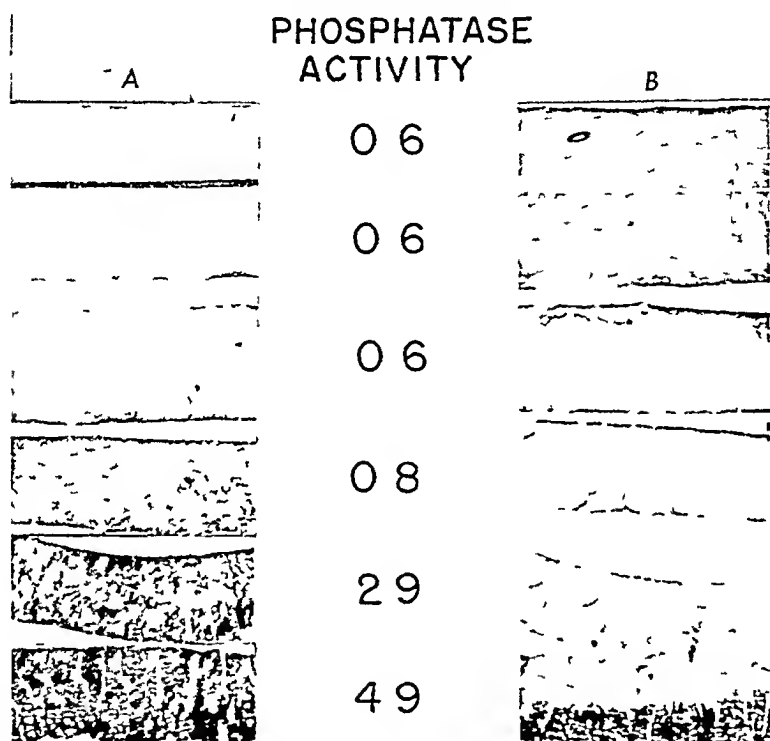


FIG. 4. Quantitative values of phosphatase activity in the costal cartilage of a child (712) compared with lower power microscopic sections from the same regions. A and B are serial sections, section B was incubated with glycerol phosphate for 2 hours. Both were then stained with silver nitrate. Note darker zone in B. Areas of lime salt deposition naturally stain black in both.

as the state of nutrition appear to have an effect on the values which have been obtained. Because of this variability, observations such as these would seem to have little significance in comparative studies of the cartilage of children. They, of course, do confirm in a quantitative way the high concentration of phosphatase at the costochondral



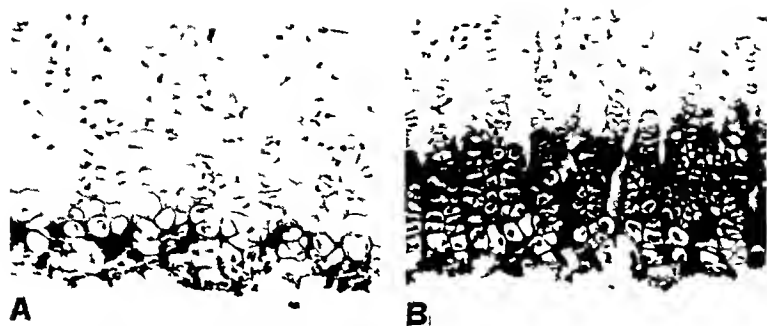


FIG 5 Higher power view of lowermost slices shown in Figure 4, A and B. Difference in dark staining material indicates presence of phosphatase activity.

TABLE III  
*Phosphatase Activity of Adult Human Periosteum*

NUMBER	AGE	SEX	P ASE ACTIVITY*	CAUSE OF DEATH
700	59	M	1 0	Bronchogenic Carcinoma
703	54	M	1 0	Carcinoma Prostate
706	57	F	1 1	Subdural Hematoma
707	24	F	1 1	Nodular Cirrhosis
708	70	F	8	Generalized Arteriosclerosis
711	43	F	7	Carcinoma Breast
715	65	F	9	Cong Heart Disease
716	35	F	1 3	Meningioma
717	30	F	1 0	Acute Pancreatitis
721	58	M	6	Glioma
723	45	F	3 5	Sarcoma Involving Vertebrae
724	69	M	3 5	Bronchogenic Carcinoma
725	60	F	1 3	Carcinoma Cervix
730	60	F	5	Carcinoma Cervix
746	42	M	2 2	Vascular Nephritis
759	63	M	6	Myeloid Leukemia
761	67	F	4	Adhesive Pericarditis
766	20	F	9	Thrombosis Portal Vein
771	54	F	6	Peritonitis

\* Expressed as Mg P Released Per Hour Per Gram Dried Tissue

junction. Values of periosteal activity in this group of children are also shown in Table II. Figure 4 illustrates the correlation between morphological differentiation, histochemical demonstration of phosphatase ac-

tivity and quantitative chemical determination of enzyme activity. Such a comparison reveals that there is chemical evidence of enzyme activity in the zone of undifferentiated cells though such may not be demonstrated by the histochemical method which has been used. Figure 5 shows the distribution of phosphatase activity by histochemical technique.

Values of phosphatase activity from the periosteum of a series of adults dying of a variety of causes are shown in Table III. With certain exceptions these values tend to be fairly uniform and group themselves about an average of 8 mg P released per gram of dry tissue per hour. The exceptions are cases No. 723, No. 724 and No. 746. The first (No. 723) was a peculiar tumor of the mediastinum with involvement of the vertebrae. The serum calcium and phosphorus values were 11.3 and 5.1 mg percent respectively. Case No. 724 was a squamous cell carcinoma with local invasion, the cause for the elevation of phosphatase activity is not clear. Case No. 746 died of chronic renal insufficiency on a vascular basis, serum calcium and phosphorus values were 7.8 and 6.6 mg percent respectively. The increased periosteal phosphatase activity in this case does not come as a surprise in view of the histological changes which may be encountered in the skeletal tissues of individuals with renal disease (4).

#### DISCUSSION

Little discussion is necessary regarding the general method which has been employed in this study. It is, of course, a variant of the technique which Linderstrom-Lang (5) has utilized so profitably. We are more fortunate, however, that, as a result of the architecture of cartilage, single slices rather than alternate ones may be used for both chemical and histological study.

Since the demonstration of alkaline phosphatase in skeletal tissues by Robison (6) in 1923, an extensive literature dealing with the rôle of this enzyme in the mechanism of calcification has accumulated. It seems unnecessary to review at this time the details of this controversial subject, recently so well covered by Moog (7) and Roche (8).

The observations which we have presented on the quantitative increase in phosphatase activity in direct relation to differentiation and maturation of cartilage cells confirm and amplify the data which have

been reported on cartilage and bone of man (9) and the rat (10) and of explanted avian bone (11) In addition they provide confirmation and place the qualitative observations obtained by histochemical techniques (12, 13) on a firm quantitative basis

Alkaline phosphatase activity in periosteum was observed histochemically by Robison (14) early in his studies on this enzyme As a matter of fact to Robison rather than to later workers should go the credit for the histochemical demonstration of phosphatase activity Other more elegant procedures on tissue sections have, of course, confirmed the presence of phosphatase activity in periosteum However to our knowledge no attention has been given to the quantitative measurement of periosteal enzyme activity We have felt that this tissue provides the purest culture of osteoblastic cells obtainable, there is little reason not to assume that strips of periosteum will reflect the activity of osteoblasts in general The reservation must be made, of course, for local increase in activity about fractures, tumor metastases, etc We are at present carrying out a series of observations on periosteal phosphatase activity in disease in order to amplify the material presented in Table III Such data, when augmented by studies on serum calcium, phosphorus concentrations and phosphatase activity should prove most interesting

#### SUMMARY

A method is described with which to correlate the morphological and chemical changes which take place in the normally differentiating costal cartilage of the dog and man

Quantitative values of alkaline phosphatase activity in costal cartilage of these two species have been correlated with the degree of differentiation of the cartilage cells

Quantitative data on alkaline phosphatase activity in the periosteum of normal dogs and children and adults coming to autopsy are presented

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# ON CREATINURIA IN MAN THE ROLES OF THE RENAL TUBULE AND OF MUSCLE MASS<sup>1</sup> <sup>2</sup>

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Urinary excretion of abnormal quantities of creatine is a recognised concomitant of a variety of pathological states. Since the ultimate composition of urine is regulated by the kidney, it is useful to consider the causes of creatinuria in terms of renal function.

Modern concepts define two factors which determine the urinary excretion of substances not secreted by the renal tubules: (1) the rate of glomerular filtration and (2) the rate of tubular reabsorption. In these terms creatinuria occurs whenever the creatine tubular load (mg creatine filtered by the glomeruli per minute) exceeds the creatine reabsorption rate (mg creatine reabsorbed by the renal tubules per minute). This disproportion might result from increased tubular load, decreased reabsorption rate or a combination of both.

Tubular load is the mathematical product of the glomerular filtration rate and the concentration of creatine in plasma; its augmentation would depend on an increase in one or both of these factors. Since significant acceleration of the glomerular filtration rate has not been observed in man in circumstances associated with creatinuria, excessive creatine tubular loads must in most instances, therefore, reflect elevated plasma creatine concentrations. Factors which might increase the quantity of circulating creatine are presented in Table 1 (I, B); essentially, these are the conditions which Hunter suggested might produce creatinuria (19).

Factors which determine tubular reabsorptive rate for creatine are little understood. The sole example of modification of this function has been demonstrated in the dog infused with glycine (32).

<sup>1</sup> Work done under a contract between the Office of Naval Research, U S Navy Department, and the Johns Hopkins University.

<sup>2</sup> Presented in part at the annual meeting of The American Society for Clinical Investigation, May 3, 1948.

Classical examples of creatinuria in man occur in such diffuse disturbances of muscle as the muscular dystrophies. However, creatinuria is associated with a number of circumstances apparently unrelated to massive muscular disease. It was the main purpose of the study reported here to determine whether or not, in these latter circumstances, modification of tubular reabsorptive activity contributed to the appearance of creatinuria. Measurements of renal function with respect to creatine were performed, therefore, under the following conditions

TABLE 1  
*Causes of Creatinuria*  
Expressed in Terms of Renal Function

	CLINICAL EXAMPLES
I Increased tubular load of creatine	
A Accelerated glomerular filtration rate	None
B Elevated concentration of serum creatine	
1 Exogenous source of creatine	Ingestion (31, 37)
2 Accelerated synthesis of creatine	Ingestion of precursors (4)
	Methyltestosterone (39)
3 Extrusion of intracellular creatine	Acute denervation atrophy (12)
	? Starvation (3)
	? Acute immobilisation (7)
4 Inadequate disposition of creatine	Muscle lack (this paper)
II Decreased tubular reabsorptive rate of creatine	
A Competitive blocking of tubular mechanism	Hyperaminoacidemia (32)
B Basis unknown	Desoxycorticosterone
	Thyroid substance
	Puerperium
	Cushing's syndrome
	(this paper)

(1) during the puerperium, (2) in thyroid disease, (a) during the early response to thyroid substance by hypothyroid subjects, and (b) in hyperthyroidism, (3) in Cushing's disease, (4) during prolonged administration of desoxycorticosterone acetate (DCA)<sup>3</sup> and (5) during administration of methyltestosterone (MT)<sup>4</sup>

<sup>3</sup> Percorten, generously supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey

<sup>4</sup> Generously supplied by Schering Corporation, Bloomfield, New Jersey

A second possible basis for creatinuria is reduction in total muscle mass leading to inadequate disposition of creatine (Table 1 I, B, 4) Prior clinical examples of this type of creatinuria are in the main inconclusive because of the coexisting factor of progressive muscle wasting As a first approximation of the role of muscle lack in spontaneous creatinuria, a study was made, therefore, of two patients with extensive loss of muscle mass due to obsolete anterior poliomyelitis

#### I CREATINURIA DUE TO DIMINISHED TUBULAR REABSORPTION

The role of the renal tubule was evaluated by simultaneous measurements of renal clearance of creatine and the glomerular filtration rate in certain circumstances associated with creatinuria

The procedures for the determination of renal function were performed with the subjects in the post-absorptive basal state Urine collections were obtained by catheterization of the patients and by voluntary voiding by the normal subjects, collection periods were approximately 20 minutes each Adequate urine flows were insured by administering water perorally A sample of venous blood was obtained once during each period In one subject from whom samples of both arterial and venous blood were obtained, creatine concentrations were identical within the limit of error of the method The concentration of the appropriate solute at the midpoint of the period was obtained by inspection of the curve formed by plotting the logarithm of the concentration of the solute in serum against time <sup>5</sup> The glomerular filtration rate was determined by calculating the clearance of inulin, mannitol or of sodium thiosulfate from the expression  $C = \frac{UV}{P}$  where C is the glomerular filtration rate (ml/min), U is the concentration of the

<sup>5</sup> Renal clearance of the endogenous chromogen giving the Jaffe reaction ("creatinine" clearance) is a function of the glomerular filtration rate Since inulin clearances were determined on the basis of a falling serum concentration, while "creatinine" clearances were determined on the basis of a constant endogenous serum concentration, variations in urine flow would be expected to disturb the "creatinine" clearance inulin clearance ratio if so-called renal delay time were a factor of practical import However, in the observations reported here, the ratio of "creatinine" clearance to inulin clearance was constant and independent of urine flow during short-term measurements in a given subject Renal delay time, therefore, has not been considered in calculation of clearances

solute in urine (mg/ml),  $P$  is its concentration in serum (mg/ml), and  $V$  is the rate of urine flow (ml/min /1.73 m<sup>2</sup> body surface)

Clearances of inulin and of mannitol were determined after a single injection. Clearances of sodium thiosulfate were determined during a constant infusion. Chemical methods were those of Harrison for inulin (15), Smith for mannitol (35) and Newman for sodium thiosulfate (27).

Creatine clearances ( $C_c$ ) were determined during rising serum concentrations before and after oral administration of 20 to 25 g of creatine. Analyses for creatine and creatinine were performed according to the method of Peters (29).

Since there is analytical error in the measurement of the rate of excretion of small amounts of endogenous creatine during acute tests of renal function, these data were checked, as a first approximation, in the case of one subject (B. R.) by expressing the creatine excretion rate, determined during an acute test, as a 24-hour output and comparing it with the known output of creatine during the day preceding the test. When it is considered that renal clearances were performed under basal conditions, these values, presented in Table 2, are in virtual agreement and support the validity of the method.

From raw data were derived the following

(1) The ratio, creatine clearance/glomerular filtration rate,  $C_c/C$ . This represents the proportion of filtered creatine which is excreted in the urine, that is, it is equal to the creatine excretion rate divided by the tubular load of creatine (*vide infra*).

(2) The proportion of filtered creatine which is reabsorbed,  $(1 - C_c/C)$ .

(3) The tubular load of creatine, or the rate at which creatine is filtered through the glomerulus  $P_c \times C$ , where  $P_c$  is the serum concentration of creatine (mg/ml).

(4) The creatine excretion rate  $U_c \times V$ , where  $U_c$  is the concentration of creatine in the urine (mg/ml).

(5) The creatine reabsorption rate, or the difference between the tubular load and excretion rate  $T_c = (P_c \times C) - (U_c \times V)$ , where  $T_c$  is the creatine reabsorption rate. The same value is, of course, obtained as the product of the tubular load and the proportion of creatine which is reabsorbed  $T_c = (P_c \times C)(1 - C_c/C)$ . Since crea-



tine reabsorption rate, particularly at endogenous loads, was calculated as the relatively small difference between two much larger terms, reliance cannot be placed on its absolute value. For this reason, tubular activity was assessed as a function of  $C_c/C$  (Table 4, column 8 and Fig. 1)

When increments in tubular load of creatine no longer result in an increased  $T_c$ , the rate of maximum tubular reabsorption of creatine, ( $T_{m_c}$ ), has been reached

Implicit in the calculation of the renal clearance of creatine is the assumption that all of the creatine in serum is free to be filtered through the glomeruli. If this assumption is incorrect, alterations in diffusibility of creatine may alter the apparent tubular reabsorption. The essential validity of the assumption, however, has been established by the demonstration that creatine was distributed uniformly between serum and certain extracellular fluids, as indicated in Table 3, and by the fact that the creatine concentration in anserobic ultrafiltrates of serum, separated by the technic of Lavietes (20), was found to equal that in serum water

In 79 urine-collection periods the endogenous "creatinine" clearance was compared to the inulin clearance. The ratio of these clearances was independent of the rate of urine flow which varied over twenty-fold and was constant in a given subject during any series of urine-collection periods. Sixty-five of the 79 clearance ratios (82 per cent) differed from their mean by no more than 10 per cent. This constancy is considered evidence for the accuracy of urine collections. It is apparent that, by the nature of the experimental procedure, inaccurate urine collections would produce divergent errors in the determination of creatine clearance (performed during *rising* serum concentration) and the glomerular filtration rate (performed during *falling* serum concentration of inulin or mannitol) and render the ratio  $C_c/C$  virtually meaningless

*Tubular reabsorption of creatine in the normal* Renal clearances of creatine were measured on four occasions in two healthy young women. The results appear in Table 6 and, in part, in Figure 1B. In agreement with the observations of Tierney and Peters (37), no creatine appeared in the urine when the serum concentration of creatine was less than 0.5 mg/100 ml, and creatinuria always occurred when the serum

concentration exceeded that value. Despite the achievement of tubular loads which were 25 to 90 times greater than the endogenous load, no  $Tm_C$  was demonstrated, i.e., the tubular reabsorptive mechanism was not saturated. The highest creatine reabsorption rate observed in each of the four experiments varied from 9.1 to 23.0 mg/min/1.73 m<sup>2</sup>. The ratio,  $C_C/C$ , tended to become constant at high creatine loads, approximately associated with concentration of serum creatine in excess of 10 mg/100 ml. In ten observations performed at these high loads, the ratio  $C_C/C$  varied from 0.55 to 0.81, with a mean of 0.68. These data are in agreement with those of Pitts (31) who compared the creatine clearance to the xylose clearance in a normal woman. When the xylose clearance is corrected to give a measurement of the filtration rate (xylose clearance = 0.75 × inulin clearance), it can be calculated from Pitts' data that his female subject had, at high tubular loads of creatine, a tubular reabsorption rate of 6 to 11 mg/min (uncorrected for body surface), and a ratio  $C_C/C$  of 0.73.

*Effect of desoxycorticosterone acetate.* B. R., Unit No. 393446, a 58-year-old male with myotonic dystrophy, a non-toxic nodular goiter, bilateral testicular atrophy, and gynecomastia, received 180 mg of DCA in oil intramuscularly, divided in daily doses for 17 days. During the first 11 days of DCA administration he retained sodium and water, gained weight, and had a less positive potassium balance. During the last six days of DCA administration he was in negative sodium and potassium balance, displayed mild polydipsia and polyuria and lost weight. Details of the electrolyte and water metabolism in this subject have been reported elsewhere (41).

Simultaneous clearances of mannitol and of creatine were determined before DCA administration and on the seventeenth day of DCA administration. The results appear in table 4. Prior to the exhibition of DCA, the ratio  $C_C/C$  rose with increasing serum concentration toward a plateau of approximately 0.8, indicating that as much as 20 per cent of filtered creatine was reabsorbed.  $Tm_C$  was approximately 3 mg/min/1.73 m<sup>2</sup> body surface. DCA induced complete inhibition of creatine reabsorption under the conditions noted. The ratio  $C_C/C$  approximated unity at all serum concentrations of creatine and  $Tm_C$  was zero (Figure 1A).

*Thyroid disease and effect of thyroid substance in hypothyroidism*

Renal clearances were measured before therapy and ten to 14 days after institution of thyroid therapy in three patients with hypothyroidism

H G, Unit No 372322, a 37-year-old housewife, had myxedema due to Hashimoto's struma, demonstrated by biopsy of the thyroid. Her BMR was -16 per cent of normal and her serum cholesterol concentration was 252 mg per cent. Two weeks after introduction of treatment with desiccated thyroid, 64 mg daily, the patient experienced

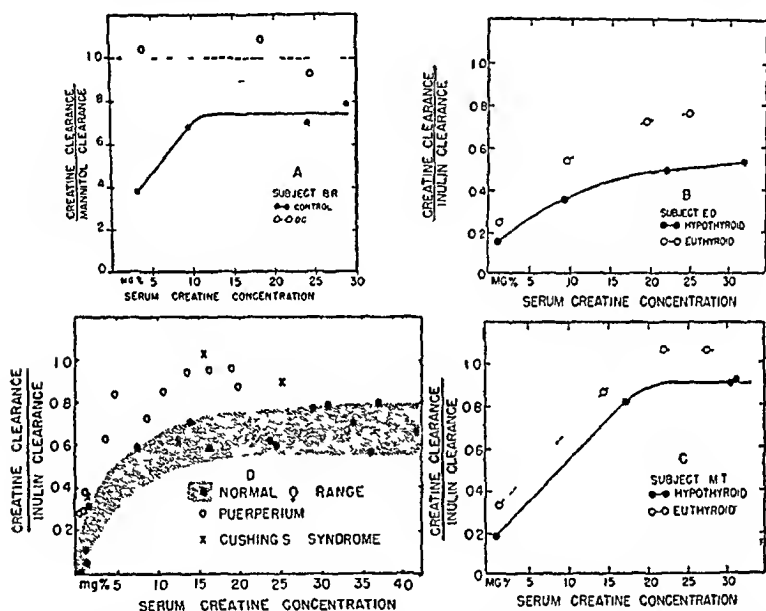


FIG 1 Relation of creatine clearance to glomerular filtration rate at various levels of serum creatine. On the ordinate a value of 0 indicates complete reabsorption of creatine, and 1.0 indicates no reabsorption.

a diuresis, her weight fell from 145 lbs to 134 lbs and dryness and puffiness of her face began to disappear. BMR rose to -4 and, ultimately, to +9 per cent of normal. Serum cholesterol concentration fell to 165 mg per cent.

E D, Unit No 366610, a 53-year-old housewife, had idiopathic hypothyroidism manifested by increasing fatigue, weight gain, dryness of skin and hair, coarse timbre of voice, dyspnea, an enlarged tongue and pitting peripheral edema. Her BMR was -27 per cent of normal.

and her serum cholesterol concentration was 328 mg per cent. With administration of thyroid substance, 96 mg daily, she improved. Her BMR rose to +2 per cent of normal and the serum cholesterol concentration fell to 215 mg per cent.

M T, Unit No 443502, a 22-year-old domestic, had hypothyroidism manifested by inability to concentrate, increasing fatigue and gain in weight. Her BMR was -25 per cent of normal and her serum chole-

TABLE 2  
*Comparison of Actual and Calculated 24-Hour Excretions of Creatine*  
Subject B R

PERIOD	G CREATINE/24 HRS /1.73 m <sup>2</sup> b s	
	Calculated	Actual
Control	0.084	0.098
DCA	0	0.015
MT (45th day)	1.253	1.365

TABLE 3  
*The Equilibrium Concentrations of Creatine in Serum Water and Extracellular Water*  
(8 patients)

CREATINE MG PER CENT		SOURCE OF FLUID
Serum Water	Extracellular Fluid Water	
0.0	0.1	Pleural
0.3	0.2	Peritoneal
0.3	0.3	Edema
0.6	0.5	Edema
0.6	0.4	Peritoneal
0.7	0.7	Pleural
0.8	0.8	Edema
1.3	1.3	Peritoneal

sterol concentration was 366 mg per cent. With administration of thyroid substance, 64 mg daily, the BMR rose to +3 per cent of normal and the serum cholesterol concentration fell to 290 mg per cent.

Exhibition of thyroid substance diminished tubular reabsorptive activity, the ratio  $C_c/C$  increased in each instance (Table 4, Figure 1B and C).

In three male subjects with hyperthyroidism tubular reabsorptive

Renal clearances were measured before therapy and ten to 14 days after institution of thyroid therapy in three patients with hypothyroidism

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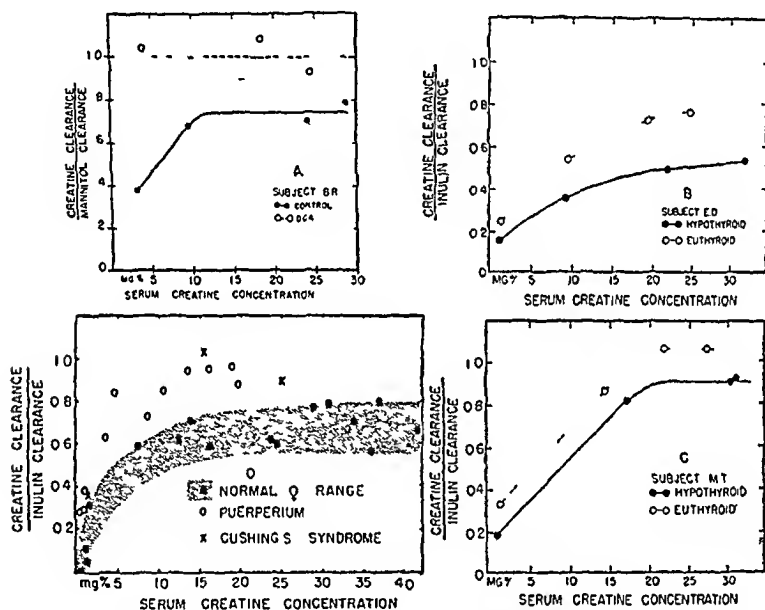


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0.6	0.5	Edema
0.6	0.4	Peritoneal
0.7	0.7	Pleural
0.8	0.8	Edema
1.3	1.3	Peritoneal

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TABLE 4  
Modification of the Renal Clearance of Creatine

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml/min) C	CREATINE				CLEARANCE RATIO CREATINE FILTRA- TION RATE Co/C
			Serum (mg %) $P_c \times 100$	Filtered (mg/min) $P_c \times C$	Excreted (mg/min) $U_c \times V$	Re- absorbed (mg/min) $(P_c \times C) - (U_c \times V)$	
1	2	3	4	5	6	7	8
M J 1 70 m <sup>2</sup>	Normal female	118 (I)	1 35	1 59	0 09	1 50	0 05
		127	7 40	9 40	5 50	3 90	0 59
		140	24 50	34 30	20 90	13 40	0 61
		110	33 80	37 20	26 30	10 90	0 71
	1 69 m <sup>2</sup>	116 3 (I)	0 93	1 08	0 32	0 75	0 30
		111 9	16 20	18 10	11 00	7 10	0 59
		111 7	31 00	34 70	27 70	7 00	0 80
		104 6	43 50	45 40	29 80	15 60	0 67
	1 69 m <sup>2</sup>	115 4 (I)	0 48	0 55	0	0 55	0
		110 3	13 70	15 10	10 80	4 30	0 71
		115 9	28 80	33 40	26 10	7 30	0 77
		132 9	37 00	48 80	39 70	9 10	0 81
D F 1 58 m <sup>2</sup>	Normal female	152 6 (I)	1 07	1 63	0 17	1 46	0 11
		153 3	23 50	36 00	22 20	13 80	0 62
		151 9	36 00	54 60	31 60	23 00	0 55
K Z 1 78 m <sup>2</sup>	Normal male	131 6 (I)	0 31	0 41	0	0 41	0
		114 3	3 65	4 17	2 14	2 03	0 51
		112 8	10 50	11 85	8 41	3 44	0 71
		119 2	17 50	20 85	13 88	6 97	0 67
J L 1 96 m <sup>2</sup>	Normal male	120 6 (I)	0 46	0 55	0 15	0 40	0 28
		140 3	6 20	8 70	4 98	3 72	0 57
		120 4	11 14	13 41	10 73	2 68	0 80
		124 3	15 60	19 40	14 35	5 05	0 74

All rates are referred to 1 73 m<sup>2</sup> body surface Creatine values are presented in hundredths of mg as determined, although the accuracy of the analytic method is limited to four parts in one hundred

(I) Inulin clearance

(M) Mannitol clearance

(T) Sodium thiosulfate clearance

TABLE 4—Continued

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml/min) C	CREATINE				CLEARANCE RATIO CREATINE FILTRA- TION RATE C <sub>C</sub> /C
			Serum (mg %) P <sub>C</sub> × 100	Filtered (mg / min) P <sub>C</sub> × C	Excreted (mg / min) U <sub>C</sub> × V	Re- absorbed (mg / min) (P <sub>C</sub> × C) - (U <sub>C</sub> × V)	
1	2	3	4	5	6	7	8
H G	1 61 m <sup>2</sup> Myxedema BMR -16	78 9*(T)	0 35	0 28	0	0 28	0
		78 9	4 10	3 23	0 82	2 41	0 25
		78 9	14 00	11 03	9 05	1 98	0 82
		78 9	22 50	17 75	14 22	3 53	0 80
	1 60 m <sup>2</sup> During thyroid treatment BMR -4	64 5*(T)	0 96	0 62	0 45	0 17	0 73
		64 5	9 00	5 80	3 87	1 93	0 67
		64 5	26 00	16 75	15 84	0 91	0 94
		64 5	38 00	24 50	22 90	1 60	0 93
	1 68 m <sup>2</sup> Myxedema BMR -25	117 8(I)	0 95	1 12	0 21	0 91	0 19
		110 8	17 10	18 93	15 45	3 48	0 82
		106 9	30 50	32 60	29 58	3 02	0 91
		114 0	31 00	35 35	32 62	2 73	0 92
	1 69 m <sup>2</sup> During thyroid treatment BMR +3	116 1(I)	1 38	1 60	0 54	1 06	0 34
		125 5	14 30	17 93	15 58	2 35	0 87
		108 3	21 80	23 62	25 07	-1 45	1 06
		114 6	27 30	31 30	33 20	-1 90	1 06
E C	1 67 m <sup>2</sup> Myxedema BMR -27	76 0(I)	1 02	0 78	0 12	0 66	0 16
		68 5	9 20	6 30	2 26	4 04	0 36
		69 5	22 00	15 28	7 57	7 71	0 49
		104 0	32 00	33 28	17 58	15 70	0 53
	1 68 m <sup>2</sup> During thyroid treatment BMR -7	115 2(I)	1 29	1 49	0 37	1 12	0 25
		85 4	9 50	8 12	4 39	4 73	0 54
		90 1	19 50	17 59	12 58	5 01	0 72
		92 6	24 90	23 10	17 50	5 60	0 76

\* Owing to unpredictable but sometimes large changes in creatine excretion with alterations in sodium excretion (42), the measurements of creatine clearance were made during four consecutive periods and then the GFR was measured in the following four periods by infusing Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>. The average of these GFR determinations was applied to the preceding periods. Justification for this extrapolation is found in the observation that, during the first experiment on subject H G, renal clearance of endogenous creatinine was not altered by infusion of Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>. Thus, during the three clearance periods immediately preceding Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> infusion, creatinine clearance averaged 86.6 (90.3, 81.6 and 88.1) and the ratio of creatinine clearance to the average thiosulfate clearance was 1.14, 1.03 and 1.12 respectively. During the three clearance periods in which Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> was infused, the creatinine clearance averaged 81.3 (76.2, 82.7 and 84.9) and the ratios of creatinine clearance to the corresponding thiosulfate clearance were 0.96, 1.05 and 1.07, respectively.



TABLE 4—Concluded

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml/min) C	CREATINE				CLEARANCE RATIO CREATINE FILTRA- TION RATE Cc/C
			Serum mg (%) Pc × 100	Filtered (mg/ min) Pc × C	Excreted (mg/ min) Uc × V	Re- absorbed (mg/ min) (Pc × C) - (Uc × V) 7	
1	2	3	4	5	6	7	8
B R	1 66 m <sup>2</sup> Myotonic dystro- phy, untreated	48 7(M)	3 19†	1 55	0 60	0 95	0 39
		56 3	9 50	5 35	3 63	1 72	0 68
		54 9	24 00	13 18	9 17	4 01	0 70
		44 7	28 70	12 83	9 91	2 92	0 77
	1 66 m <sup>2</sup> During DCA	68 6(M)	9 10†	6 25	6 52	-0 27	1 04
		83 9	18 50	15 53	17 00	-1 47	1 10
		50 2	24 30	12 21	11 26	0 95	0 92
	1 66 m <sup>2</sup> During methyl- testosterone (45 days)	67 5(I)	4 35	2 94	0 91	2 04	0 31
		68 3	5 00	3 42	1 35	2 07	0 40
		73 8	13 00	9 60	8 76	0 84	0 91
		56 8	24 60	13 97	10 32	3 65	0 74
	1 65 m <sup>2</sup> During methyl- testosterone (50 days)	71 3(I)	4 40	3 14	1 09	2 05	0 35
		84 1	13 00	10 93	6 81	4 12	0 62
		55 9	25 50	14 27	13 59	0 68	0 95
		60 2	44 50	26 79	19 05	7 74	0 71
D L	1 69 m <sup>2</sup> Cushing's disease	97 3(I)	1 24	1 21	0 43	0 78	0 36
		96 0	12 40	11 90	7 27	4 63	0 61
		86 5	15 60	13 50	13 97	-0 47	1 04
		86 8	25 40	22 05	19 80	2 25	0 90
M S	1 37 m <sup>2</sup> Post partum 5th day	160 0(I)	0 75	1 20	0 34	0 86	0 28
		170 0	3 36	5 72	3 61	2 11	0 63
		179 0	14 00	25 05	18 10	6 95	0 72
		166 0	19 90	33 02	29 05	3 97	0 88
H D	1 81 m <sup>2</sup> Post-partum 5th day	113 8(I)	1 03	1 17	0 44	0 73	0 38
		110 0	8 60	9 46	6 92	2 54	0 73
		116 8	16 60	19 40	18 43	0 97	0 95
		117 8	19 00	22 39	21 50	0 89	0 96
S C	1 65 m <sup>2</sup> Post-partum 4th day	165 6(I)	0 96	1 59	0 46	1 13	0 29
		167 8	4 70	7 90	6 65	1 25	0 84
		162 6	10 60	17 23	14 58	2 65	0 85
		166 8	13 60	22 65	21 40	1 25	0 94

† These periods do not represent endogenous levels but levels following the ingestion of creatine. In all other experiments the first period represents measurements made just before ingestion of creatine.

activity was normal with respect to creatine. Renal clearance determinations, repeated in two subjects when they had become euthyroid, revealed no change in the ratio  $C_c/C$ . In each subject during his hyperthyroid phase endogenous creatine concentration in serum was greater than normal for the male, in agreement with the observations of Tierney and Peters (37), and was reduced with treatment. Creatinuria in hyperthyroidism, then, may be the result of increased tubular load, an end result of several possible pre-renal changes (Table 1, IB).

*Puerperal creatinuria* It has long been known that a profound creatinuria accompanies the first week or two of the puerperium. At one time it was held that puerperal creatinuria was the result of release of creatine from the involuting myometrium (34), but it has been calculated that more creatine is excreted in several days post-partum than is contained in the entire gravid uterus (2). Renal clearances were measured in three subjects on the fourth or fifth day post-partum. The three subjects, Unit Nos 437362, 402467, 409667, all had had normal, full-term pregnancies with uneventful labors and uncomplicated post-partum periods. All were lactating. Data on renal performance appear in table 4 and figure 1D, together with data on a group of normal subjects. The ratio  $C_c/C$  during the puerperium well exceeded the normal. Furthermore, since the concentration of endogenous creatine in the serum was essentially normal while the ratio  $C_c/C$  was elevated, puerperal creatinuria appears to be the result of reduced tubular reabsorption of creatine. It will be noted that in two of the three subjects GFR was greater than the normal range, creating an increased tubular load. However, this increase would not produce creatinuria of the degree displayed by these subjects if the efficiency of tubular reabsorption of creatine were normal. At maximum tubular loads, creatine reabsorption rate was only a fraction of normal.

*Lack of effect of methyltestosterone* MT, 50 mg daily, was administered orally to the myotonic subject B R for 30 days, during which time creatine excretion rose from zero to more than 1400 mg a day. During the peak of creatinuria simultaneous clearances of inulin and creatine were measured on two occasions.

MT was without effect on tubular mechanisms for creatine reabsorption (Table 4), but was accompanied by a rise in serum creatine to a concentration of more than 4 mg per cent, at which level creatinuria

of the same order occurs in the normal subject who has received a large dose of creatine by mouth (Figure 1D)

*Cushing's disease* D L, Unit No 479544, a 26-year-old singer, complained of deepening of her voice, thinning of her scalp hair, acne of face and arms, increase in body hair, gain in weight with especial roundness of her face Her appearance was typical of Cushing's disease She had abdominal striae, hirsutism and enlargement of the clitoris Her blood pressure was 165 mm Hg systolic and 105 mm Hg diastolic There was slight osteoporosis of the lumbar spine Fasting blood glucose concentration and glucose tolerance were normal Serum sodium concentration was as high as 152.5 mEq/l She excreted increased amounts of 17-ketosteroids (32-39 mg/day), but not of 11-oxysteroids (0.4 mg/day) There was eosinopenia (0 - 55 per cu mm)

Data on renal performance appear in table 4 The ratio  $C_c/C$  was greater than normal, and at maximum tubular loads the creatine reabsorption rate was less than half of the lowest normal value

## II CREATINURIA ASSOCIATED WITH REDUCED MUSCLE MASS

Although it has been proposed for about 30 years (19) that a reduction in available muscle storage space forces creatine to overflow into the urine, the clinical material which has been studied does not afford a clear demonstration of this possible cause of creatinuria Relevant reports have dealt with states in which muscle atrophy was progressive (23, 24) In these states it may be considered that dissolution of muscle adds creatine to extracellular fluid and that a portion of this increment may appear in the urine Determination of the creatine content of the urine, in such cases, does not permit a decision as to whether creatinuria results from extrusion of intracellular creatine or from inadequate disposition of newly-synthesised or ingested creatine In order to isolate one of these factors from the other, there were selected for study of this problem two patients with extensive muscular atrophy due to obsolete anterior poliomyelitis, in whom the possibility of progressive repair or destruction of muscle was remote The assumption was made that the remaining muscle cells retained normal function The clinical state of these patients precluded the complete study of renal function which was planned The fragmentary observations

which were made are recorded here, nevertheless, because they support an hypothesis concerning one mechanism responsible for certain instances of creatinuria

L R , Unit No 398745, a 24-year-old white female, had been bed-fast for eight years following an acute episode of anterior poliomyelitis in 1938 She weighed 60.8 kg and her surface area was 1.60 m<sup>2</sup> Flaccid paralysis was complete in all extremities, thorax, trunk and neck Breathing was entirely diaphragmatic Although calculi were demonstrable roentgenographically in the right kidney, no evidence of impaired renal function was obtained Arterial pressure was 110 mm Hg systolic and 70 mm Hg diastolic On one occasion a few leucocytes appeared in the urine, on other occasions urinalysis was normal At the end of two hours 45 per cent of an intramuscular dose of phenol-sulphonphthalein was recovered in the urine Blood non-protein nitrogen concentration was 20 mg per cent There was no evidence of abnormal catabolic activity, the patient had no recent weight loss, fever or leucocytosis, and her basal metabolic rate was -21 per cent of normal

When preliminary analysis revealed creatinuria, nitrogen balance was assayed to determine whether or not the creatinuria in this bed-fast patient might be similar to that reported to occur during acute immobilisation (7) The patient was placed on a constant diet calculated to contain 50 g of protein, 58 g of fat, 117 g of carbohydrate and 1200 calories per day Included in this diet were 50 g of meat Identical menus were offered each day An aliquot representing one-half of one day's ration of each five-day batch of food was analysed for nitrogen by a macro Kjeldahl method (5) Pooled five-day stools were dried on a steam bath and analysed for nitrogen Urine was collected daily under 10 ml of 25 per cent acetic acid and analysed for nitrogen and guanidoacetic acid (8)

The results of these determinations appear in Table 5 There was a profound creatinuria and a marked reduction in creatinine excretion The concentration of creatine in the serum was elevated to 1.6 mg per cent That this increase in tubular load of creatine was not the result of extrusion of creatine from the intracellular mass and was unlike the creatinuria observed in acute immobilisation is suggested by the presence of positive nitrogen balance on a moderate nitrogen in-

take (0.8 g of protein per kg body weight) With certain reservations to be discussed below, the normal excretion of guanidoacetic acid is considered to be compatible with a normal velocity of synthesis of creatine. A conservative estimate of tubular activity may be made by assuming that the glomerular filtration rate was normal and that the post-absorptive concentration of creatine in the serum prevailed throughout the day. The amount of creatine filtered, then was approximately  $100 \text{ ml/min} \times 0.016 \text{ mg/ml} \times 1440 \text{ min/day} = 2304 \text{ mg/day}$ . Of this quantity, 714 mg appeared in the urine on the day on which the serum was obtained. The proportion of creatine which was reabsorbed was, therefore,  $\left(1 - \frac{714}{2304}\right) \times 100$ , or 69 per cent, which

TABLE 5

*Daily Excretion of Creatine Bodies and Nitrogen Balance in Obsolete Poliomyelitis*

DAY	URINE			
	Nitrogen Balance	Guanidoacetic	Creatinine	Creatine
	g	mg	mg	mg
1-5	+1.59	48.5	216	925
6-10	+0.85	42.7	211	904
11-15	+1.48	54.4	226	893
16-20	+0.57	—	212	965

Subject L. R. Body weight, 60.8 kg. Nitrogen intake 8.1 to 8.3 g/day by analysis. All values are expressed as averages for 24 hours. Serum creatine concentration, 1.6 mg per cent.

is a low normal tubular performance at a serum concentration of 1.6 mg per cent.

At the completion of the balance periods, subject L. R. was placed on a creatine-poor diet and, after equilibrium to the new intake had occurred, a creatine tolerance test was performed by the technic of Thorn (36).

A second subject was observed similarly on a creatine-poor diet. This subject, B. A., Unit No. 240356, a 28-year-old white female, developed acute anterior poliomyelitis in 1941 with initial flaccid paralysis of the thorax and all four extremities. Following recovery from the acute phase, her activity was slightly less restricted than was that of the previous subject, and she regained about 60 per cent of normal function in the right upper extremity.

The data on urinary excretion of creatine and creatinine during creatine-poor intake appear in Table 6. Both subjects displayed pronounced creatinuria and a reduced excretion of creatinine. Creatine tolerance was severely impaired.

If the assumptions are correct, it would appear that mere loss of muscle mass is an adequate cause of creatinuria in obsolete anterior poliomyelitis, on the principle that creatine, synthesised at normal velocity and lacking adequate muscle space for its disposition, overflows into the urine. A plausible derivative is that creatinuria may be expected as a non-specific phenomenon wherever there is sufficient muscle wasting, provided that synthesis and reabsorption of creatine proceed at normal rates.

TABLE 6  
*Urinary Excretion of Creatine Bodies in Obsolete Anterior Poliomyelitis*

	NORMAL (60 KG B W) FEMALE	SUBJECT	
		L. R.	B. A.
Creatinine (mg /day)	840-1300	180	304
Creatine (mg /day)	0-60	714	588
Creatinine coefficient	14-22	3	5
Total creatinine coefficient	14-22	13.3	13.2
Creatine tolerance (per cent)	70	10	27

It is interesting to note that over a period of years no measurable adjustment in rate of creatine production was made in the face of diminished peripheral requirement for creatine.

#### DISCUSSION

*Causes of creatinuria* in terms of renal function have been listed in Table 1. Measurement of glomerular filtration rate (GFR) and of renal clearance of creatine will determine whether a given instance of creatinuria is the result of increased tubular load, decreased tubular reabsorption rate or both. If the creatinuria is associated with decreased reabsorption rate, presently available technics do not permit further differentiation. If creatinuria results from an increased tubular load some differentiation may be made between its two components: GFR and the serum concentration of creatine. It is unlikely that in spontaneous disease an increase in GFR occurs in sufficient mag-

nitide to cause significant creatinuria, in most instances increased tubular load will reflect elevated concentration of serum creatine

Indirect assessments of *velocity of creatine synthesis* in man have been made by following the fate of isotopic creatine (18) and by measuring the urinary excretion of guanidoacetic acid (4) The isotopic technic is refined but not easily applied to clinical study at this time With respect to guanidoacetic acid it remains to be demonstrated that renal tubular mechanisms do not modify its urinary excretion Until this possibility is studied conclusions regarding creatine synthesis must be tentative <sup>6</sup>

*Extrusion of intracellular creatine* might be expected to operate when a significant number of muscle fibers disrupt as, for example, during rapid atrophy in acute anterior poliomyelitis In the presence of negative metabolic balances of nitrogen, phosphorus, potassium and sulfur, in ratios peculiar to their relative concentrations in muscle protoplasm, it may be surmised that creatine similarly escapes the cell It is conceivable, too, that under certain circumstances creatine might escape from muscle cells without accompanying leakage of other cellular constituents It is not possible presently to dissect these mechanisms free from other concurrent factors which may be operating to produce creatinuria One of these factors is considered immediately below

*Inadequate disposition of creatine* It is reasonable to suppose, on the basis of the observations reported here on subjects with obsolete anterior poliomyelitis, that creatinuria occurs when reduction in muscle mass is great enough to render residual tissue insufficient for proper disposition of creatine Essential to this supposition is the maintenance of normal velocity of synthesis and rate of tubular reabsorption The assumptions used in the calculations above appear reasonable, but the observations do not preclude the possibility that a minor fraction of the creatine excreted resulted from slight diminution in tubular reabsorption

It is possible that a minor reduction in muscle mass does not cause creatinuria In such cases deposition of creatine may occur in liver, kidney, skin or remaining muscle since these tissues will absorb large

<sup>6</sup> Sims and Seldin have observed that tubular reabsorption of guanidoacetic acid diminishes as the tubular load of creatine increases (34a)

amounts of extra creatine in the rat (6) Such alternate deposition apparently may be limited in man For example, in a patient who had undergone an amputation of the leg and half the pelvis for sarcoma we have observed an elevated serum creatine concentration and significant creatinuria three weeks post-operatively when the patient was afebrile, ambulatory and had exhibited no obvious loss of remaining muscle

*Speculations on the lack of creatinuria in myotonic dystrophy* There are some striking exceptions to the observation that creatinuria accompanies reduction in muscle mass myotonic dystrophy (9), certain instances of hyperthyroidism (28) and, perhaps, hypoadrenalism (11, 40) In a subject with reduced muscle mass absence of creatinuria might result from increased tubular reabsorption, augmented metabolism of creatine by alternate pathways or decreased velocity of synthesis These possibilities have been surveyed in the case of myotonic dystrophy

Lack of creatinuria in myotonic dystrophy is not due to increased tubular reabsorption In two patients with severe muscular wasting due to this disease the concentration of creatine in serum was normal (zero to 0.4 mg per cent) Appropriate measurements of renal functions in one of these patients (B. R., Table 4) indicated that creatine was reabsorbed with normal efficiency Two observations suggest that there is not significant metabolism of creatine along pathways alternate to its conversion to creatinine, (1) the pattern of rising concentration of creatine in the serum after an oral dose of creatine was normal and (2) a profound hypercreatinemia was achieved and maintained by administration of MT

There remains the possibility that the velocity of creatine synthesis is decreased in myotonic dystrophy It is concluded from the response to MT that the *capacity* to synthesise creatine is not defective In the light of this observation it may be suspected that some undefined stimulus normally concerned with the full production of creatine is lacking in myotonic dystrophy

*Significance of tubular reabsorption of creatine* The renal tubules of normal adult man on a creatine-poor diet reabsorb some 560 mg of creatine each day (assuming a serum creatine concentration of 0.003 mg/ml and a GFR of 130 ml/min) These tubules are capable,



however, of reabsorbing completely a load of some 1000 mg of creatine per day (renal threshold for creatine is approximately 0.005 mg/ml (37), GFR is 130 ml/min (10), and  $1440 \text{ min/day} \times 0.005 \times 130 \times 1440 = 936 \text{ mg/day}$  or  $0.65 \text{ mg/min} / 1.73 \text{ m}^2 \text{ b s}$ ). Since creatinuria of the order of but 100 mg per day is considered significant, a reduction of only 10 per cent of the capacity for reabsorption will result in notable creatinuria. Thus, when the rate of creatine reabsorption is depressed creatinuria may appear or increase. An example of such depression in the dog has been reported by Pitts (32) who effected a reduction in creatine reabsorption by infusing glycine, and concluded that  $\alpha$ -amino acids competed for the same tubular transfer agency. In man, depression of creatine reabsorption has been observed during administration of DCA to patients with myotonic dystrophy, of thyroid substance to patients with hypothyroidism, in a patient with Cushing's syndrome, and spontaneously during the puerperium. In none of these conditions is there a rise in the serum concentration of  $\alpha$ -amino acids sufficient to account for the creatinuria, if the phenomenon is comparable in man and dog. This does not exclude the possibility that the creatinuria accompanying diabetic acidosis, for example, results in part from the significant hyperaminoacidemia which occurs (21).

There is no readily apparent causal factor common to the various instances of depression of creatine reabsorption which have been described here. In the case of DCA, collateral evidence suggested that widespread reduction in general tubular reabsorptive activity had occurred (41). This was indicated partly by the mild diuresis which accompanied the creatinuria. Such mild diureses accompany the early period of therapy in the hypothyroid patient and the early puerperium. That water diuresis alone does not induce creatinuria has been established in this laboratory by producing wide variations in urine flow, both acutely and over 24-hour periods, without altering creatine excretion or reabsorption (42).

Data presented in this report indicate that when thyroid substance was exhibited to patients with hypothyroidism an early effect was decreased tubular reabsorption of creatine. Wilkins and Fleischmann (38) observed that, with respect to urinary excretion of creatine and creatinine, the effects of thyroid treatment of myxedematous patients

fell into two phases. During the first phase the output of creatinine was not changed greatly, but creatine excretion increased so that the total output of creatine and creatinine exceeded that of the hypothyroid state. In the second phase, the total output of creatine and creatinine was approximately equal to that of the hypothyroid state or perhaps slightly less. In this phase creatinine excretion was decreased and creatine excretion increased compared to the levels before treatment. Our observations were made during the first phase. It was at this time that the subjects studied by Wilkins and Fleischmann were largely in negative nitrogen balance. Synchrony between negative nitrogen balance and creatinuria is frequent. It occurs in starvation (3), during acute immobilisation (7), and during convalescence from acute illnesses (13). Wilkins and Fleischmann considered that in two of their subjects the nitrogen deficit was too slight to account for the increments in creatinuria in terms of dissolution of protoplasm. It is possible, therefore, that the parallel changes in nitrogen excretion and creatinuria are in some measure the common result of depression of renal tubular reabsorption. While the data at hand warrant no conclusion concerning the mechanism of creatinuria in starvation, immobilisation and convalescence, these states may be associated with the process called adaptation (33) which has been linked with adrenal cortical activity. That adrenal corticoids influence renal tubular reabsorption of creatine is suggested by the observations reported in the patient with Cushing's disease and in the case of administration of DCA. Whether or not the creatinuria accompanying the first few weeks of thyroid administration in myxedema and the creatinuria of the puerperium are examples of adaptation cannot be discerned from the evidence at hand. It may be recalled simply that during the early puerperium profound homeostatic adjustments are in full course in many systems.

*Relationship of the creatinine coefficients to renal function.* The constancy of the creatinine coefficient (mg creatinine excreted in 24 hours/kg body weight) has been emphasised repeatedly, a narrow range of values is found for normal men (20-26) and for normal women (14-22) (40). It has been held on reasonable grounds that, since the main source of creatinine is the creatine of muscle, the creatinine coefficient is a function of muscle mass. However, the observations reported here

on the modification of creatine excretion effected by the kidney suggested the necessity for further analysis of the renal factor in creatinine excretion. Endogenous creatinine is not reabsorbed actively by the renal tubule. Because tubular secretion of true endogenous creatinine is virtually insignificant in normal man, endogenous creatinine clearance may be an approximate measure of the glomerular filtration rate (26).<sup>7</sup> Thus, the amount of creatinine excreted is determined largely by the GFR and by the plasma concentration. A reduction in either of these factors might decrease the creatinine coefficient. With diminishing GFR, but with maintained muscle output of creatinine, the serum creatinine rises, e.g., renal insufficiency. Conversely, if renal function is maintained but muscle output is diminished serum creatinine falls. Examples of this latter situation were found in three patients with hyperthyroidism in whom the serum concentrations of true creatinine were low but then rose during treatment concurrently with a return of body weight and muscle bulk, without significant alteration of GFR. Thus, it would appear that in most cases an estimate of muscle mass derived from the value of the creatinine coefficient must presuppose a normal GFR, and, conversely, an estimate

<sup>7</sup> The concentration of true endogenous creatinine in serum may be approximated from the expression  $P = \frac{UV}{C}$ , when  $P$  is the serum concentration of true endogenous creatinine,  $UV$  is the rate of urinary excretion of creatinine (mg/min) determined by the Jaffe reaction, and  $C$  is the GFR measured by inulin, mannitol or thiosulfate clearances. The assumptions inherent in this expression are dependent upon the observations that (a) true endogenous creatinine clearance is an approximate measure of GFR (1, 26) and (b) the preformed Jaffe chromogen in urine, in contrast to serum, is virtually all true creatinine (25). When  $P$  was calculated in this fashion in 84 clearance periods in man, true creatinine was 73 per cent (range 51 to 107 per cent) of the Jaffe chromogen in serum. The same data indicate that the renal clearance of endogenous Jaffe chromogen is, on the average, only 73 per cent of the GFR, so that, as determined by the method at hand, the Jaffe chromogen clearance is not a measure of the GFR. The data indicate that the non-creatinine component of Jaffe chromogen is either not diffusible through the glomeruli or is diffusible and subsequently reabsorbed completely by the renal tubules. The latter possibility is supported by the fact that in eight patients with accumulations of serous or edema fluid the mean concentration of endogenous Jaffe chromogen was 1.99 mg per cent in serum water and 2.00 mg per cent in other extracellular water.

of renal function by measurement of the serum concentration of creatinine must assume a normal output of creatinine from the muscles

Since Shaffer's original studies (34) it has been stated by some observers that the *total* creatinine coefficient (mg creatine expressed as creatinine + mg preformed creatinine excreted per day/kg body weight) in subjects with creatinuria equaled the creatinine coefficient in the normal (14, 22). This concept is difficult to reconcile with present knowledge of the differing modes by which the kidney treats creatine and creatinine. Since creatine is reabsorbed by the renal tubules, while creatinine is not, equimolar loads of creatine and creatinine would not result necessarily in equimolar excretion of these two substances. On these grounds it would be anticipated that the *total* creatinine coefficient would be reduced in patients with a diminished creatinine coefficient and a spontaneous creatinuria. A review of reported data on the excretion of total creatine bodies by subjects with creatinuria reveals, in fact, that the total creatinine coefficients were below the normal range. For example, in seven adult males with progressive muscular dystrophy and copious creatinuria on a creatine-free diet, total creatinine coefficients ranged from 13.0 to 18.3, compared to the normal range of 20-26 (23). And in the two patients with obsolete anterior poliomyelitis reported here the total coefficients were less than those reported for normal women. It would appear, then, that inasmuch as creatine is reabsorbed, the total creatinine coefficient in creatinuric patients will be less than the creatinine coefficient of normal subjects. And variations in reabsorption rate may underlie the inconstancy of the total creatinine coefficient reported by many observers.

These considerations render unproved the assumption made by some observers (17) that the sum of daily excretion of creatine, creatinine and guanidoacetic acid equals the amount of creatine synthesised.

#### SUMMARY

1) Creatinuria may be considered to be the result of an increased tubular load of creatine (serum creatine concentration  $\times$  glomerular filtration rate), decreased tubular reabsorption rate, or a combination of both. On theoretical grounds, tubular load may be augmented by

acceleration of (GFR), by administration of creatine, accelerated synthesis of creatine, extrusion of intracellular creatine, or by inadequate disposition of creatine in the muscle cells

2) The ability of the renal tubule to reabsorb creatine was reduced and creatinuria was observed (a) during prolonged administration of desoxycorticosterone acetate and in a patient with Cushing's disease, (b) during administration of thyroid substance in instances of hypothyroidism, and (c) during the puerperium. The creatinuria accompanying the administration of methyltestosterone to man had no renal tubular component.

3) In obsolete anterior poliomyelitis, creatinuria followed simple reduction in muscle mass, an example of inadequate bulk for proper disposition.

4) By isolation of some of the mechanisms concerned with the production of creatinuria, it appeared that the anomalous absence of creatinuria in patients with myotonic dystrophy may be the result of reduced synthesis of creatine.

5) The creatinine coefficients and the serum concentration of creatinine are functions not only of the active muscle mass but also of the glomerular filtration rate.

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# PRELIMINARY OBSERVATIONS ON THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) IN ALLERGIC DISEASES

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In April, 1949 Hench and his collaborators (1) described the dramatic effect of cortisone and adrenocorticotrophic hormone (ACTH) in patients with rheumatoid arthritis. In a subsequent report (2) suggestive results were obtained with cortisone in acute rheumatic fever. In one case ACTH was administered in small doses for a period of seven days. Fever subsided within forty-eight hours and articular symptoms within four days.

The experimental studies of Rich and Gregory (3, 4) suggested a relationship between the hypersensitive state and the rheumatic diseases. The possible usefulness of ACTH in the control of the hypersensitivity state was first explored in a patient with severe exfoliative dermatitis due to iodine. The unique rapidity of recovery in this critically ill patient stimulated the trial of ACTH in other allergic states.

In the second patient extensive giant urticaria, joint pains and fever associated with a "serum disease type" sensitivity to penicillin were abolished within twenty-four hours after ACTH was begun.

The prompt control of the chronic asthmatic state and the striking alterations in the tissues of the upper respiratory tract in five asthmatic patients form the basis of this preliminary report.

In two patients the asthma was thought due to combined external and intrinsic factors. In the other three it was of the intrinsic type. The age of the patients ranged from 26 to 63 years, and the duration of the asthma from 5 to 23 years. The sputum in all contained many eosinophils. In several of the patients no more than partial and very temporary relief was obtained by the administration of adrenalin, aminophyllin, and ether by rectum.

The initial daily dose of ACTH varied between 30 and 100 mgm given intramuscularly, divided in equal portions at six hour intervals. Unequivocal benefit was noted in from four to forty-eight hours. Complete freedom from all asthmatic symptoms occurred within one to eight days. In four of the patients there was total disappearance of sputum, and abnormal physical signs in the chest. In the fifth patient, who was six months pregnant, rhonchi persisted although she was free of asthmatic symptoms. Coincident with symptomatic relief, tracings of the expiratory phase of respiration showed improvement with removal of the relative obstruction to outflow.

Treatment was maintained for eleven to twenty-one days. The daily dose was gradually reduced after clinical recovery, the total amount administered ranging from 360 to 775 mgm. One patient has remained asymptomatic for one month after therapy was stopped.

Detailed examinations of the upper respiratory tract, including nasopharyngoscopy, were made on four of the patients. Three patients had a pale, edematous, polypoid nasal mucous membrane which was bathed with a thick mucopurulent discharge. During therapy the membrane became bluish pink in color, was covered with clear mucus, and the edematous, polypoid appearance was no longer present. The breathing space was greatly enlarged. The lymphoid tissue in the nasopharynx, which was covered with a thick discharge, was pale and edematous. During treatment the edema subsided, an orange pink color developed, the crypts became more prominent, and it was easily outlined from the surrounding mucous membrane. There was no gross change in the volume of the lymphoid tissue present. In two patients there was complete obstruction of the nose by polyps. These began to shrink on the third day of treatment and by the end of therapy had completely vanished in one and almost completely in the other. The three cases with mucous membrane abnormalities had antral clouding on roentgenological examination which cleared during the administration of ACTH. Several small polyps had reappeared in one patient on the twenty-third day after cessation of therapy.

The intradermal reactions to inhalant and bacterial antigens were followed in three patients. Two showed marked skin sensitivity to pollens and other extrinsic antigens. In one the sensitivity was greatly diminished during treatment but returned to its original level three weeks after cessation of ACTH. In the other patient no alteration was

observed In both patients serum reagin titration revealed no change The intradermal reaction to bacterial antigens decreased significantly in two patients and did not change in another In one the sensitivity has returned to the pretreatment level three weeks after discontinuance of ACTH

The previously described (1, 2) metabolic effects of ACTH in no instance were a serious complicating factor in these cases

These clinical studies suggest that ACTH may have an important action in blocking various hypersensitivity reactions Further observations are necessary to establish its efficacy in allergic states, and the effects of ACTH in other types of hypersensitivity are under study

The ACTH used in these studies was supplied by Armour and Company through the courtesy of Dr John R Mote

We wish to thank Dr Walter Baetjer, and Dr Milton Sherry for permission to treat patients under their care

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## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

*The Commonsense Psychiatry of Dr Adolf Meyer* BY ALFRED LIEF XVIII + 677 with a frontispiece, glossary and index *McGraw-Hill, New York, 1948 \$6 50*

Under this title, Mr Lief has presented an anthological biography of Dr Adolf Meyer,—a book, largely in Dr Meyer's own words, in which one can trace the development of his thought and teaching, with sufficient biographical material to provide personal perspective

Large numbers of students and associates who knew Dr Meyer as "the Professor," have now this solid, attractively printed, volume with which to re-view many matters originally viewed as difficult or incomprehensible In this review many will find themselves a bit puzzled to understand why it was ever so mystifying Mr Lief has wisely chosen those central themes of Dr Meyer's developing thought around the ever-recurring, deeper problems of psychiatry and medicine, and those who now re-read his words, with a mature medical experience as background, may therefore find them more understandable, more exciting, more cogent, than they seemed when originally heard or read

Another large group of readers will also find this an extremely useful and stimulating book—the great number of present-day psychiatrists to whom Dr Meyer has been a symbol often referred to but not so often read Even those who have spoken or written glibly about "Adolph Meyer" and who profess to have read his (non-existent) "textbook of psychiatry" can now achieve an approximate honesty by reading this book

Your reviewer, in reading Mr Lief's book, has gained a clearer perspective of Meyer as essentially a biologist with a special interest in constructing a broad basis for a biological "science of man" For this general purpose, comparative neurology was Meyer's "structural-functional" framework, and in clinical psychiatry, "the experiments of nature" were daily "scrutinized" for better understanding of "man-function" The ergasia-formulations may be taken symbolically to represent Dr Meyer's preference for a dynamic biological (Greek-rooted) functional frame of reference, and his aversion to a static (Latin-rooted) pigeon-holing orientation for clinical psychiatry

The title to Mr Lief's book somewhat unpleasantly implies a proprietary claim to "common sense," as if other psychiatric views and practices did not partake of "common sense" Your reviewer might undertake to dispute the aptness of this implication if it were not so difficult to define "common sense" when used as a laudatory adjective There is a deeper meaning, however The "consensus" was an inherent part of Dr Meyer's mode of operations He sought a "consensus" with each patient, he sought a "consensus" with his staff, with other earnest work-

ers in the broad fields of psychiatry and neurology, and this everlasting search for common ground and mutual agreement was an outstanding characteristic of Meyer's work pattern—the Meyergasia, so to speak.

In this sense of a searching for the sense of things, and searching for a sense which could be agreed upon in common, "common sense" is an apt methodological characterization of Adolf Meyer's method of psychiatric work.

JOHN C WHITEHORN

*Synopsis of Psychosomatic Diagnosis and Treatment* By FLANDERS DUNBAR, M D  
501 pages C V Mosby Company, St Louis, 1948

Dr Dunbar's reputation, the excellent bibliography, and the handy format make this book very tempting. However, as poor organization, poor selection of material, and faulty editorship are the outstanding features, the reader's attention must be directed to the excellent chapter on metabolic and endocrine disorders written by Dr Robert C Lowe, and the highly competent portions devoted to gastrointestinal disorders and arthritis.

The book is not a synopsis. It is a collection of original articles mixed with a peculiar array of incomplete chapters. Some topics are covered in considerable detail while other important subjects are either mentioned in passing or are entirely omitted. Some chapters have little place in a book with this title and were better eliminated, particularly when it has been found necessary to confine all remarks on general therapy and rehabilitation to four pages.

The book is introduced by a chronology of fetal development, its pertinence is purely hypothetical and of little interest to the reader of a synopsis. The second chapter by L W Sontag is an intriguing and scholarly presentation of determinants of predisposition to psychosomatic ailments, the emphasis on genetic and intra-uterine influences is not only purely hypothetical, but out of keeping with most of the literature including the chapters which follow in the book. The third introductory chapter concerning the nervous system seems to the reviewer to be a poor and uncritical selection of data.

The editorship of the clinical chapters is puzzling. For example, the chapter entitled "Vasomotor, Trophic, Oral, and Skin Disorders" confines itself to skin ailments and completely neglects such conditions as Raynaud's Syndrome, migraine, and causalgia. The chapter on "Eye, Ear, Nose, Throat, and Dentition" devotes twenty pages and the only illustrations in the volume to disorders of dentition, most of which is not only trivial but of little interest to the internist, but does not mention the nose and throat, although the author must be aware of the work on emotional influences on the nasal mucosa being carried on at Cornell. There are many other strange inconsistencies.

The book can be recommended for the few good chapters with the hope that the remainder will not turn the reader from the field which Dr Dunbar had previously done so much to further.

T L

*Geriatric Medicine The Care of the Aging and the Aged* 2nd Edition E J STEIGLITZ, EDITOR 773 pp W B Saunders, Philadelphia, 1949 \$12 00

Forty-seven experienced authors have contributed 44 chapters to this text-book which covers the fields of medicine and some aspects of special surgery. The aim of this symposium is to enhance the "application of understanding of the changes which come with senescence to all the facets of medical practice". The need for such an effort is beyond argument, there is some ground, however, to question whether a text of this nature is the best solution to the problem.

There are some introductory chapters on general principles of the aging process which make available a certain number of valuable quantitative data diluted by a large quantity of generalisations, aphorisms and slogans which are presented in extended form. In some sections the paucity of specific information is disappointing, in others, many clinicians would disagree vehemently with the advice given. For example, in a brief discussion of sleep, the only sedatives mentioned are phenobarbital and sodium pentothal, failure to mention barbital and chloral hydrate is difficult to understand when the drugs recommended here are notorious for their capacity to produce confusion in the elderly patient.

A second criticism may be directed toward the inclusion in this book of articles which, although superb clinical treatises, are not of special geriatric interest. For example, Talbott's chapter on gout and Keefer's on diseases of the aorta are magnificent essays which appear to be forced into the book for purposes of completeness. In view of the rapidly mounting costs of medical texts, it would seem important that authors and editors strive to reduce rather than expand the size of their books.

It is somewhat dismaying to find in a text of 773 pages devoted to geriatric medicine that "Mental Changes with Normal Aging" is dealt with in a matter of 14 pages and "Mental Disease" in another 15 pages. By contrast, "Surgical Renal Disease" occupies 16 pages, "Diseases of the Prostate" 18 pages and "Diseases of the Bones" 31 pages.

Despite certain deficiencies noted, this text provides in one source a large quantity of information related to geriatric medicine which is disseminated throughout the literature. It will furnish a starting point for those requiring an introduction to gerontological problems and is to be recommended as a preliminary reference text.

J L L, Jr

*Cardiac Catheterization in Congenital Heart Disease* ANDRE Cournand, JANET S BALDWIN AND AARON HIMMELSTEIN 108 pp \$4 00 Published by the Commonwealth Fund, New York

The book of Cournand and his co-workers is primarily concerned with non-cyanotic malformations of the heart. Its general section deals with the technique, equipment and with complications of the method. This section also includes characteristic blood pressure tracings and formulae used in the calculation of the blood

flow through various parts of the circulation Cournand is well equipped to discuss this procedure since he was the first to use catheterization as a diagnostic tool in this country, and to standardize the technique so that it may be safely used for diagnosis and investigative purposes Several points in the general section of the book deserve mentioning The authors perform catheterization in children under 9 years of age under basal avertin anesthesia Other workers prefer morphine or scopolamine Cournand and his co-workers use a modified Hamilton manometer and also mention the more popular electrical recording devices According to Cournand, the most frequent complications of catheterization in Congenital Heart Disease are, air emboli, venous and intracardiac thrombosis and cardiac arrhythmias The latter is the most serious complication Fortunately, however, the combined deaths from the procedure, reported from various laboratories is probably less than 1 percent The pressure recordings are excellent and should be of the greatest value to all those who are interested in the diagnosis of Congenital Heart Disease The formulae for the calculation of the various volume flows are similar to those used in other laboratories

In the second section of the book Cournand and his co-workers deal with the application of catheterization to specific malformations of the heart and great vessels The correlation of clinical and physiological findings is of particular value, since it enables the authors to establish patterns for the more typical forms of Congenital Heart Disease

Cournand's book stresses the fact which has been so often overlooked in the past that a similarity in gross pathology is often associated with a large variety of physiological and clinical findings The book should be considered essential to all those who are interested in the application of modern physiological methods to the study of Congenital Heart Disease

RICHARD J BING

*Practice of Allergy* By WARREN T VAUGHN, M D Revised by J Harvey Black, M D, Second Edition, 1132 pp Illus \$15 00 C B Mosby Company, St Louis, 1948

The present writer had the opportunity of reviewing the first edition of this book published in 1939 The second edition necessitated by ever-increasing knowledge in the allergic field was interrupted by Dr Vaughn's untimely death, and Dr J Harvey Black undertook the difficult task of completing the revision Dr Black tried, as he states, to "retain the quality and the flavor of the book so that it might remain as it was written—Warren Vaughn's book " Except for completely new and good sections on pollen field surveys and the development and technique of Aerobiology by O C Durham, Chief Botanist of the Abbott Laboratories, a complete revision of the chapter on fungus infection and associated allergies by Dr J B Howell, and a chapter on vital capacity and pulmonary physiology by Dr James Holman, the general text remains essentially as Dr Vaughn originally wrote it in 1939

The historical aspects of the development of our present understanding of

clinical allergy and the discussion of the general characteristics of clinical allergy are followed by individual sections devoted to the physiology of allergy, allergic diagnosis, diagnosis and treatment of food allergy, food allergens, pollens and other inhalent antigens, bacterial allergy, fungi, serum disease, anaphylactic shock, drug allergy, contact allergy, physical allergy, the pharmacology of allergy and finally the specific allergic syndromes as met with in human beings

Dr Vaughn possessed an encyclopedic mind and the subject matter is presented in an interesting manner. His style, however, is somewhat rambling, repetitious and at times speculative in character. Dr Vaughn was particularly interested in special phases of allergy which are given a disproportionate amount of space. One hundred and sixty-one pages are devoted to the diagnosis and treatment of food allergy, the importance of which in clinical allergy has been seriously questioned by many physicians in recent years. Food allergy as a common cause of asthma, chronic rhinitis, migraine, colitis and hypertension has aroused skepticism in many minds. Of the prominent allergists in this country, Dr Vaughn was an expert botanist and the detailed section of two hundred and sixty-eight pages devoted to plants and pollens reflect his interest and knowledge.

The treatment of hay fever of the seasonal type reflects the fact that the immunologic mechanism conferring protection on the individual is not yet known, and therefore, the appropriate dose of pollen extract for any given individual cannot be foretold. The discussion of perennial rhinitis, a more serious affliction though less common than seasonal hay fever in which aetiology and successful therapy is more difficult to obtain receives only minor consideration. Likewise, the section on urticaria and angioneurotic oedema, especially of the chronic type should be amplified. Only twenty-four pages are devoted to asthma whereas thirteen pages are devoted to migraine, which in the experience of this reviewer is only rarely due to food allergy. The disproportionate emphasis on special subjects should be eventually corrected.

Certain sections could be combined to prevent repetition, for example, anaphylactic shock and serum disease could be included in the first section which covers the historical development of anaphylaxis and clinical allergy. The section on clinical characteristics of allergy (Part II) could be combined with allergic diagnosis (Part IV). There is, however, much good material in this book reflecting Dr Vaughn's wide clinical experience as stated in the review of the first edition ten years ago. The many case analyses, the numerous good photographs taken by the author with a Lecca camera lend a personal touch not usually encountered in most text books. Numerous portraits of early investigators are also of interest. The bibliography is extensive but not complete. The printing, on good grade paper, is easy to read, a recommendation when one considers the size of the book.

A textbook of over 1000 pages on a special phase of medicine necessarily must contain a great amount of detail, to the extent that this volume serve more as a reference book than a concise text book for medical students and physicians who are not too familiar with the minutiae of allergy.



*Advances in Pediatrics, vol 3* Edited By S Z LEVINE and others 363 pp \$7 50  
*Published by Interscience Publishers, Inc New York*

This volume contains eight excellent articles written by experts Five of the articles are factual reviews of the subjects covered These are of timely interest and a storehouse of information to be used either for teaching or for meeting a specific practical problem in diagnosis or therapeutics The other three articles are of a different nature, for in them the contribution is that of point of view of the author himself All three deal with some phase of development Lawson Wilkins' article deals with sexual development, and although much is concerned with physiological and anatomical abnormality, there is an excellent discussion of the variations in "normal" development, a discussion much needed in these days of commercialized pressure to use endocrine drugs of doubtful efficacy or safety, whenever the patient varies even a little from the average

Hilde Bruch's article on puberty and adolescence is an original contribution of first rate importance, amounting to much more than a review of recent advance In it, she has reviewed an entire subject including anthropological, sociological and other aspects of puberty, as well as making her own contribution to an understanding of the psychology of this period

Milton Senn's article while in general a good presentation of the psychological factors in childhood development, tends too much to dogmatic statement He illustrates a fault not too uncommon among psychiatrists of generalizing on the basis of "slanted material"—the psychiatrist seldom sees a "normal" child, except his own In spite of these somewhat minor "defects" the article is a good presentation of present points of view in child psychology

H W J

*Hematology for students and practitioners* Revised 2nd edition WILLIS M FOWLER  
535 pp \$8 50 *Paul B Hoeber, Inc, New York, N Y*

This compact volume is designed to present in concise form a resume of clinical hematology for the medical student and practitioner The major portion of the text is concerned with descriptions of the blood dyscrasias However, there are brief sections dealing with basic anatomical and physiological concepts and a chapter describing routine hematologic methods The volume is not intended as a reference work and does not deal exhaustively with the subject matter discussed A bibliography is provided at the end of each chapter, but specific references to the literature are not cited in the text The book is clearly written, is very readable and contains a wealth of accurate and well condensed information The author presents fairly both sides of many controversial points, but here and there unqualified statements appear which certainly would not meet general approval For example, the normal intravascular fluidity of the blood is attributed to the presence of heparin In another section, one is led to believe that leukemia can be transmitted from man to animals In view of our present knowledge of iron me-

tabolism it seems regrettable that the term "idiopathic" hypochromic anemia has been retained. For the most part, however, the book is up-to-date and is very adequate for the purpose for which it was written.

C L C

*Clinical Cystoscopy* By LOWRAIN E. MCCREA. 2nd ed. 1152 pp. 742 illus. (201 in color) \$28.00. F. A. Davis Company, Philadelphia, 1949.

This is an enlargement of an already proven first edition authoritative text on cystoscopy. There is a presentation of every phase of cystoscopy and related urological fields so that in fact it becomes almost an omnibus of urology. The two volumes are beautifully printed, and the illustrations on almost every page add clarity and understanding to the well presented and well documented text. The enlarged and improved index and the profuse bibliography are helpful in attaining the desired end—a ready, useful reference book.

The author's use of his excellent cystoscopic photographs, in which he has pioneered, is interesting and instructive. However, the cystoscopic drawings are often clearer, and one wonders if a more careful use of post mortem material might not be more satisfactory. The text and value of the book often suffer from the attempt to include a little bit about everything in cystoscopy and related urology, sometimes at the expense of fundamental detail. At times the author is of necessity didactic and sometimes on controversial subjects. Unfortunately, some of the drawings, which are copies of familiar originals suffer in the transfer, and one wonders why the originals were not used.

The two volumes undoubtedly cover the field of clinical cystoscopy more completely and carefully than any other available text. Their breadth of scope and their usefulness as reference material for the unusual as well as the commonplace findings of cystoscopy should recommend them highly to any student of urology and particularly to the neophyte who is trying to master the intricacies of the subject.

W E G

*Atlas of Peripheral Nerve Injuries* WILLIAM R. LYONS AND BARNES WOODHALL. 339 pp. \$16.00. W. B. Saunders, Philadelphia, 1945.

This monograph presents the pathologic changes of peripheral nerves as they have been seen in war casualties of World War II treated in Army general hospitals during the years 1943, 1944 and 1945.

After a brief summary of the pathologic techniques used in the preparation of the tissues, and a glossary of the terms employed, the authors describe briefly the normal histological structure of peripheral nerve. The incidence of periphery nerve injuries in World War I and II is discussed with particular reference to the surgical treatment.

By means of hundreds of photographs, some in color, of gross and histological specimens, the authors illustrate the changes in the proximal and distal stumps of

severed nerves, the alterations in neuromas in continuity, and the effects of ischaemic lesions on the peripheral nerve and muscle. The outstanding feature of this book is the profuse use of illustrations with accompanying legends and pertinent comments of interest to all workers in the field of neurology.

Although a representative bibliography is appended, little attempt is made to integrate the contents of the monograph with pre-existing knowledge of peripheral nerve pathology. Perhaps the voluminous material at the disposal of the authors seemed sufficiently great to make superfluous, comments on opinions based on fewer cases. Many readers will wish that the literature had been more fully discussed.

As an atlas, this is a superb contribution. Its format and printing are pleasing to the eyes. But one could not help wishing that the end results in the cases had been known, so that the effect of the various pathological states described might be correlated with the functional restoration of the nerve. We may hope that the exigencies of civilian life do not place too many obstacles in the way of the authors, so they may prepare, as they announce in an addendum, a separate monograph on the influence of the neuropathologic changes described in this atlas upon peripheral nerve regeneration.

A E W

## BOOKS RECEIVED FOR REVIEW

- A Textbook of Neuropathology* BEN W LICHTENSTEIN 474 pp \$9 50 W B Saunders, Phila Pa
- Change of Life* EMIL NOVAK 127 pp \$2 00 Women's Press, New York
- Diagnosis of Pancreatic Disease* LOUIS BAUMAN 74 pp \$5 00 J B Lippincott, Phila Pa
- Epilepsy and Convulsive Disorders in Children* EDWARD M BRIDGE 670 pp \$8 50 McGraw-Hill Co, New York
- Mental Hygiene in Public Health* PAUL V LEMKAU 396 pp \$4 50 McGraw-Hill Co, New York
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- Radiologic Exploration of the Bronchus* S DI RIENZO 332 pp \$10 75 Charles C Thomas, Springfield, Illinois
- Shearer's Manual of Human Dissection, 2nd edition* CHARLES E TOBIN 286 pp \$4 50 The Blakiston Co, Phila Pa

## LOW ELECTRICAL SKIN RESISTANCE IN THE REGION OF PAIN IN PAINFUL ACUTE SINUSITIS\*

THOMAS E. VAN METRE, JR.†

*Psychobiological Laboratory Phipps Psychiatric Clinic, and the Department of Medicine,  
Johns Hopkins University Medical School, Baltimore 5, Maryland*

Received for publication October 6, 1949

Objective signs of pain are of obvious importance, particularly when communication with the patient is impossible or untrustworthy. Such signs may be found by careful examination of accessible areas of referred pain in visceral disease. Here it may be possible to map out areas of hyperaesthesia, muscle spasm, abnormal sweating, vasomotor changes, and areas of increased pilomotor activity (1). Any technic which makes this examination more delicate should be useful. Such signs as abnormal sweating, vasomotor changes, and increased pilomotor activity suggest the existence of sympathetic hyperactivity in the region of referred pain. Study of electrical skin resistance offers an extremely sensitive method for demonstrating areas of sympathetic hyperactivity (2). This method will pick up changes in sympathetic activity which the physical senses of the examiner cannot. It might be useful, therefore, in the examination of areas of referred pain.

Painful sinusitis affords an ideal testing ground for determining the value of the skin resistance method in studying areas of referred pain. The disease can be diagnosed accurately. Definite pain is referred to the surface of the body. Various therapeutic procedures give relief from pain without interfering with the skin of the pain reference area. It is possible, therefore, to obtain accurate measurements of skin resistance before and after relief of pain.

It was decided, therefore, to study electrical skin resistance in the region of pain in painful acute sinusitis. This is a preliminary report containing observations on nine patients.

\*Carried out under a contract between the Office of the Surgeon General of the U S Army and The Johns Hopkins University.

†Captain, MC, U S Army, stationed at Heidelberg, Germany.

forth between areas of high and low resistance at successively smaller intervals, it was possible to determine quite accurately the point at which the resistance dropped. This point was marked with a skin marking pencil. This process was repeated on other parts of the face until by joining all of the points together the boundary of the entire area of low resistance could be defined. Then the area of low resistance was explored using a similar rheostat setting to make sure that the area

FACIAL PATTERNS OF LOW SKIN RESISTANCE  
(DOTTED) IN NORMALS

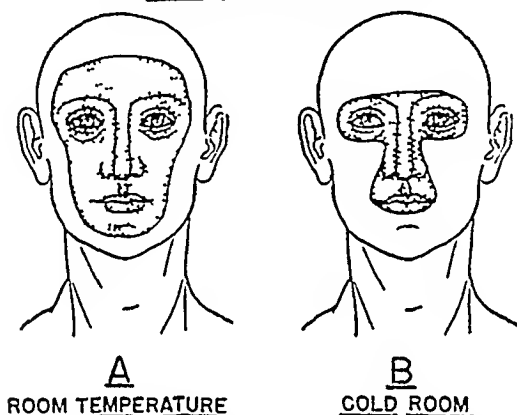


FIG 1 Facial pattern (stippled) of low electrical skin resistance in normal individuals

A Shows the large type of pattern seen when the subject is in a warm environment or when excited

B Shows the constricted type of pattern seen when the subject is in a cold environment (55°F). The pattern may become even smaller and involve only the mouth and lips. These normal patterns are invariably symmetrical.

contained no islands of high resistance. Finally, the rheostat was adjusted so that the current from the batteries scarcely deflected the ammeter pointer when the electrode was at the outskirts of the area of low resistance. The low resistance area was then further explored in order to detect areas of still lower resistance. This procedure was continued until the high, intermediate, and low resistance areas of the entire face had all been mapped.

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## MATERIALS AND METHODS

Skin resistance was measured with a 22.5 volt dermatometer (3) in the manner described by Richter and Woodruff (4)

The dermatometer circuit consisted of a 22.5 volt battery, a rheostat to regulate the voltage applied to the patient and a microammeter which registered change in resistance of the skin. This was connected to the patient by means of two electrodes. One of these electrodes was painted with Cambridge Electrode Jelly and secured to the patient's ear lobe. This ear lobe had been previously punctured with a needle in the area of attachment of the electrode. Therefore, this electrode was in contact with the highly conductive fluids of the body. The second electrode was movable and could be brought into contact with the skin of any portion of the body. Because of the attachment of the first electrode to the conducting fluids of the body, the resistance of the skin beneath this movable electrode was the resistance measured by the dermatometer (2).

In these experiments the dermatometer was not used to measure actual resistance, but rather to indicate changes in resistance as the movable electrode was moved from place to place on the skin. The current from the batteries was so adjusted that when the movable electrode was placed over areas of high resistance, the ammeter pointer moved no more than a few divisions on the dial. Consequently, when the electrode was placed over areas of lower resistance, the pointer moved further across the dial. By using the rheostat to regulate the current properly it was possible to determine the points of highest skin resistance, the points of lowest skin resistance, and the points of skin resistance which were at various intermediate levels between the two extremes.

The skin resistance pattern of the face was mapped out by the following procedure. The movable electrode was placed in contact with the skin of the upper part of the forehead, which has a comparatively high resistance. The rheostat was set so that the current from the batteries scarcely deflected the ammeter pointer. Then the movable electrode was moved towards the center of the face, touching the skin at points one-half inch apart. A close watch was kept on the ammeter pointer for a sharp deflection which would show that an area of low skin resistance had been reached. By moving the electrode back and

forth between areas of high and low resistance at successively smaller intervals, it was possible to determine quite accurately the point at which the resistance dropped. This point was marked with a skin marking pencil. This process was repeated on other parts of the face until by joining all of the points together the boundary of the entire area of low resistance could be defined. Then the area of low resistance was explored using a similar rheostat setting to make sure that the area

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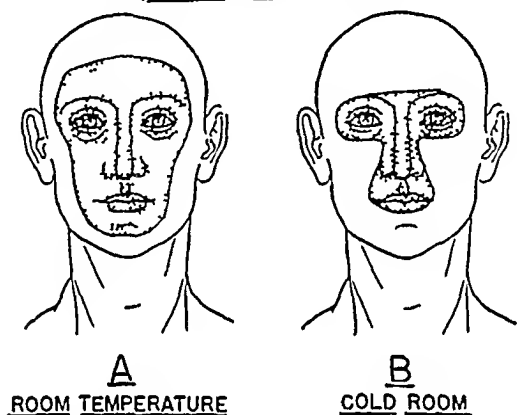


FIG 1 Facial pattern (stippled) of low electrical skin resistance in normal individuals

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contained no islands of high resistance. Finally, the rheostat was adjusted so that the current from the batteries scarcely deflected the ammeter pointer when the electrode was at the outskirts of the area of low resistance. The low resistance area was then further explored in order to detect areas of still lower resistance. This procedure was continued until the high, intermediate, and low resistance areas of the entire face had all been mapped.

The skin of the normal face shows a sharply demarcated symmetrical

area of low electrical resistance which usually includes both eyelids, the nose, the mouth, and varying portions of the forehead, of the cheeks, and of the skin of the lower lip. This area varies in size according to the external temperature (Fig 1). In warm environments the area of low skin resistance may dilate to include the skin of the entire face. In cold temperatures the area becomes progressively smaller until it includes only the mouth and lips. This area is called the normal facial pattern of low skin resistance (4).

A large normal facial pattern of low skin resistance often overlaps the area of referred pain. It could interfere with the detection of abnormal low skin resistance in the painful area. Therefore, the patients were cooled in a cold room (55°F) or with an electric fan in order to reduce the normal facial pattern to the smallest possible size. By use of this procedure it was possible to remove the normal facial pattern from the area of referred pain.

Using the methods described above, the facial skin resistance pattern was determined in nine consecutive cases of acute sinusitis which were seen at the Johns Hopkins Hospital, Baltimore, Maryland, and the General Dispensary, U S Army, Pentagon Building, Washington, D C.

Since morphine and most other analgesics have a marked effect on the electrical skin resistance, the patients were examined before they received any form of medication.

## RESULTS

An abnormal area of low skin resistance was found in the region of pain in each of the nine consecutive cases of painful acute sinusitis. These abnormal low resistance areas disappeared after the pain went away in the seven cases that were followed.

Fig 2 shows these results in more detail. The drawings labeled 'A' show the typical distribution of pain in each case (solid black shading). This is entirely typical of the type of sinusitis involved. The drawings labeled 'B' show the low facial skin resistance patterns while pain was present. These (stippled) asymmetrical patterns have two components: first, the symmetrical normal facial pattern of low skin resistance; second, a sharply demarcated area of low skin resistance that in some manner is associated with the pain. It may be con-

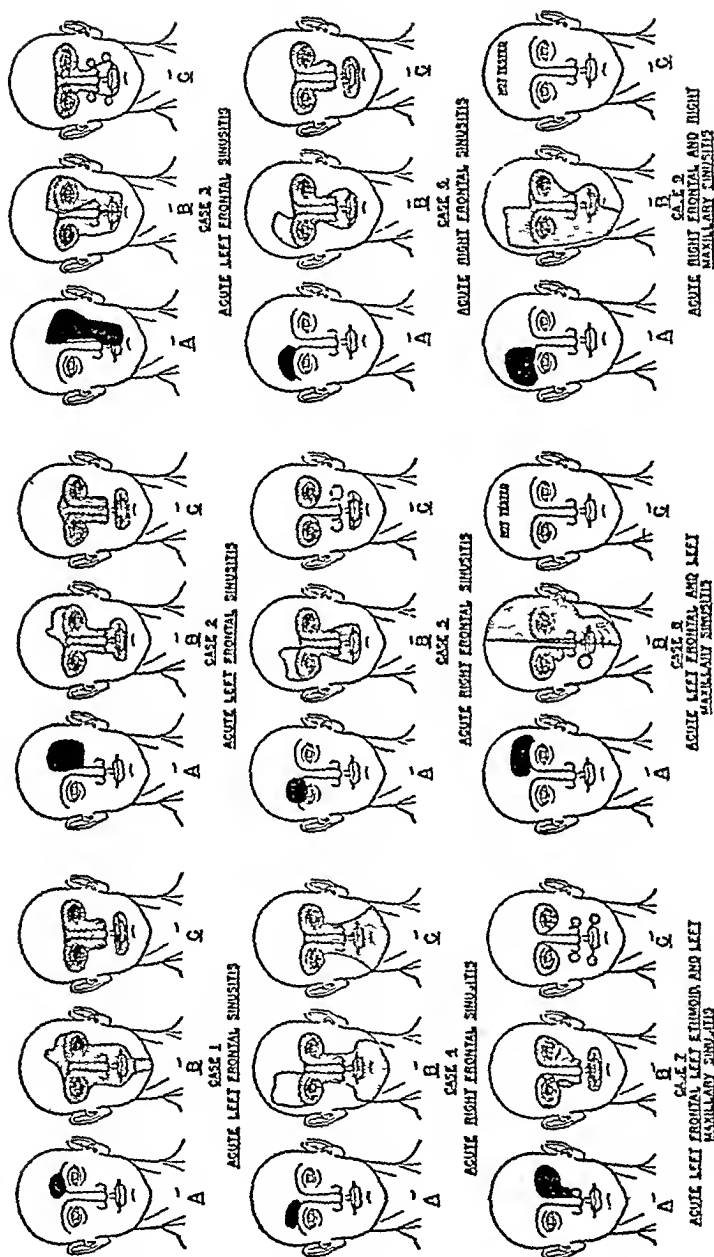


FIG 2 Data on the 9 cases of acute sinusitis. In each instance drawing 'A' shows the distribution of pain, drawing 'B' shows the electrical skin resistance pattern while pain was present, and drawing 'C' shows the skin resistance pattern after pain had disappeared.

tiguous to or partially superimposed on the symmetrical facial pattern. The resistance in this painful area may be equal to (case 8,9), lower than (case 3, 7), or higher than (case 1, 2, 4, 5, 6) the resistance found in the symmetrical facial pattern of low skin resistance. The drawings labeled 'C' show the low skin resistance patterns after pain had disappeared (stippled). These patterns are all normal symmetrical facial patterns of low resistance. The abnormal areas of low skin resistance seen in drawing 'B' have disappeared along with the pain. It is of interest that although all of the mappings on an individual patient were done under the same external environmental conditions, the symmetrical facial pattern of low skin resistance is small when pain is absent. Sinus pain, therefore, seems to cause two skin resistance phenomena: (a) an abnormal area of low skin resistance which is located in the region of pain and (b) a symmetrical enlargement of the normal facial pattern of low resistance.

Physical examination detected evidence of sympathetic hyperactivity in only one patient. In case 8, visible hyperhydrosis was noted in the region of pain and abnormal skin resistance. This patient was not followed and consequently it is not known what happened to the hyperhydrosis after relief of pain.

#### DISCUSSION

The findings reported above have not been observed in studies of the facial skin resistance patterns in a very large number of patients without acute sinusitis. Therefore, it is reasonable to presume from the data advanced that abnormal areas of low skin resistance can be found in the region of pain in acute painful sinusitis. It is probable that this area of low skin resistance is due to sympathetic hyperactivity in the region of referred pain. In favor of this possibility is the large body of evidence showing that the resistance of intact skin varies with sympathetic activity, particularly of the sweat glands, being highest when sympathetic activity is low or is eliminated by sympathectomy, and being lowest when sympathetic activity is high (2). The visible hyperhydrosis present in the area of pain in case 8 may be further evidence of local sympathetic hyperactivity. However, the lack of information on what happened to this patient after pain was relieved limits the value of this evidence.

These areas of abnormal low skin resistance in the region of sinus pain may prove to be of considerable value in the diagnosis of sinusitis. However, further studies will be necessary first to indicate (a) whether such areas can be found in all painful cases of sinusitis, (b) whether they are present in cases of painless sinusitis, (c) whether they may be present in cases with facial pain due to causes other than sinusitis. Additional investigation is called for to determine whether these findings in referred sinus pain will be present in referred pain from any viscus.

#### CONCLUSIONS

An abnormal area of low skin resistance was found in the region of pain in each of nine consecutive cases of painful acute sinusitis. In the seven cases that were followed these low resistance areas disappeared after the pain was removed.

#### REFERENCES

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- 4 <sup>1</sup> RICHTER, CURT P. AND WOODRUFF, BETTYE G. Facial Patterns of Electrical Skin Resistance: Their Relation to Sleep, External Temperature, Hair Distribution, Sensory Dermatomes, and Skin Disease. Johns Hopk. Hosp. Bull. 70: 442-459, 1942.



# AGGLUTINATION OF RED CELLS ALTERED BY THE ACTION OF NEWCASTLE DISEASE VIRUS\*

## I THE EFFECT OF CHICKEN SERA FROM INFECTED BIRDS ON SENSITIZED CELLS

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The immediate response of a host in adjusting to an invading virus is not yet understood, so that changes in the sera in the acute phase of an infection are of particular interest. Burnet and Anderson in 1946 (1) described a phenomenon in which human red cells, previously "sensitized" by the virus of Newcastle Disease of chickens, were then agglutinated by the sera of patients having infectious mononucleosis. They suggested this might indicate a serological relationship between the two diseases. Although agglutination of Newcastle-sensitized cells by sera from cases of infectious mononucleosis has not been consistently demonstrated in this country, the peculiar ability of certain sera from other diseases to agglutinate these sensitized cells has been demonstrated (2). It was also shown by the Australian workers that an agglutinin of sensitized chicken cells was present in the sera of chickens infected with Newcastle Disease. We have studied particularly the phenomenon in the chicken and have defined the conditions under which it occurs. It is a property of serum taken early in the course of active disease and not in the convalescent stage.

"Sensitization" of either human or chicken red cells is achieved by exposing a concentrated cell suspension to the action of a suspension of Newcastle virus in an incubator for 2 hours\*. During this time virus has presumably been adsorbed on and subsequently released from the red cells. The cells are then washed 3 times in saline. A suspension of these cells may be shown to be altered in that they will no longer be

\*Supported in part by a Grant in Aid from the National Institutes of Health, U S P H S

†The original description of Burnet and Anderson calls for 1/2 hour, but in our experience 2 hours produced more stable cells

agglutinated by a second exposure to the virus. These sensitized cells can, however, be agglutinated by certain sera which do not agglutinate normal cells.

In this paper we will consider the agglutination of sensitized chicken cells by sera from acutely infected chickens and the factors which influence this agglutination. In a later paper we will consider the agglutination of human "O" cells by human sera and similar factors influencing this agglutination.

The results fall into three general groups. First is an evaluation of the method of performing the test, secondly the study of the time during the illness of the chicken when the test is positive, and thirdly an attempt to study some of the characteristics of the substance present in chicken sera causing the agglutination.

#### METHOD

In the performance of the actual test, normal cells obtained at the same time from the same bird, and which have received the same treatment except for exposure to virus, are run as controls. Except where specifically indicated, tests were run at 4°C. One of the variables in the test is the preparation of stable sensitized cells, i.e. cells which will not spontaneously agglutinate. Preliminary experiments indicate that the time of shaking, the degree of aeration, and perhaps exposure to glass, all influence this stability.

Since the virus titer of the supernatant fluid after incubation equals the titer of the original virus suspension (3), and since no definite virus particles have been seen by electron microscopy to adhere to the red cells (4), it may be assumed that few intact virus particles remain on the red cells.

*Preparation of Sensitized Cells*—We have two strains of Newcastle virus under continuous study in the laboratory. One of these ("B") is relatively avirulent, but is an excellent agglutinator of chicken red cells. The other, (CG179), though highly virulent for 8-week old chickens, is a poor and inconstant agglutinator of red cells (5). Recently in connection with the effect of the virus on human cells, we have obtained from Dr. A. S. Evans the original Australian strain of virus used by Burnet and Anderson.

A comparison of the sensitivity of cells treated by these three strains is presented in Table I. All three strains of virus did sensitize cells as shown by their reactivity with various sera. In general the strain of virus producing the highest

titer of agglutination of red cells was the bset sensitizing virus, and the poorest agglutinator produced the least sensitive cells

The degree of adsorption of the virus on the red cell might influence the outcome. A comparison of the adsorption of the three strains of virus on chicken

TABLE I  
*Sensitizing Effect of Three Different Strains of Newcastle Disease Virus*

	CELLS SENSITIZED BY			
	#	B	Cg	Australian
Chicken Sera Acute Phase of Newcastle Disease + Sensitized Chicken Cells	1	80	10	
	2	2560		640
	3	160		40
Human Sera + Sensitized Human "O" Cells (Atypical Pneumonia)	1	160	80	
	2	10	0	
	3	10	2	
Chicken Cell Agglutinin Titer of Virus Preparations Used		1280-2560	0-20	320

All figures refer to highest dilution causing 2+ agglutination

TABLE II  
*Effect of Exposure of Varying Dilutions of Freshly Harvested Virus to a One Per Cent Suspension of Washed Chicken Cells for Varying Periods of Time*

STRAIN OF VIRUS	DILUTION OF ALLANTOIC FLUID EXPOSED	CONTROL	20 MINUTES	40 MINUTES
B* (agglutinator)	1/10	9 2	8 8	
	1/100	8 7		8 5
Cg (poor agglutinator)	1/10	8 5	8 3	
	1/10	8 7	7 7	8 5
Australian	1/10	9 3	9 3	8 7
	1/100	9 3	7 3	

\* Data for this strain previously published (5)

All figures refer to the log of the 50% infectivity end point

cells fails to show any correlation with the sensitivity of treated cells. This may however be due to the crudeness of the infectivity titrations, which are probably not accurate below 0.6 log.

It is indicated later that some positive human sera lost titer on remaining at 4°C. This loss is less in chicken sera. However, we have

frozen all sera in order to preserve it Freezing and thawing had little apparent effect on the chicken sera (Table III)

During the course of our experiments with human sera it was found that tests which were run at 4°C yielded much higher titers than those

TABLE III  
*Effect of Freezing for 6 Days and Thawing on Acute Phase Sera Titer*

CELLS FOR CHICKEN %	SERUM TESTED AFTER	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
#37 Sensitized	24 hours in										
	Deep freeze -16°C	+++	+++	+++	+++	+++	+++	+	0	0	0
	Quick freeze	+++	+++	+++	+++	+++	+++	+	0	0	0
	CO (-70 C)	+++	+++	+++	+++	+++	+++	+++	+	0	0
	Icebox (4 C)	+++	+++	+++	+++	+++	+++	0	0	0	0
	6 days in										
	Deep freeze -16 C	+++	+++	+++	+++	+++	+++	+++	0	0	0
	Quick Freeze	+++	+++	+++	+++	+++	+++	+	0	0	0
#37 Normal	CO	+++	+++	+++	+++	+++	+++	+++	+	0	0
	Icebox	+++	+++	+++	+++	+++	++	+	0	0	0
	6 days in										
	Deep Freeze	0	0	0	0	0	0	0	0	0	0
	Quick Freeze	+	0	0	0	0	0	0	0	0	0
	CO	+	0	0	0	0	0	0	0	0	0
	Icebox	0	0	0	0	0	0	0	0	0	0
	6 days in										
#73 Sensitized	Deep freeze	+++	+++	+++	+++	+++	+++	++	0	0	0
	Quick freeze	+++	+++	+++	+++	+++	+++	+++	++	0	0
	CO <sub>2</sub>	+++	+++	+++	+++	+++	+++	++	+	0	0
	Icebox	+++	+++	+++	+++	+++	+++	++	+	0	0
	24 hours in Icebox	0	+++	+++	+++	+++	+++	+++	++	0	0

TABLE IV  
*Effect of Temperature on Agglutination of Sensitized Chicken Cells\**

TEMPERATURE	DILUTION OF ACUTE PHASE SERA								
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
4°C	+++	+++	+++	+++	++	+	0	0	0
20-23°C	++	++	+++	+++	+	0	0	0	0
35°C	+++	+++	++	+	0	0	0	0	0

\* All of same dilutions were tested against normal cells and were negative throughout

run at approximately 25°C A study of the effect of temperature on the test with chicken sera vs sensitized chicken red cells is presented in Table IV As may be seen the effect is minimal

In an attempt to find some of the variable factors in the tests we tabulated the results of the same sera against cells from different chick-

TABLE V

*Effect of Source of Chicken Red Cells on the Titer of Various Sera*

Figures in Table refer to the Final Dilution of Sera which produced a 2+ Agglutination of Sensitized Cells

INDIVIDUAL SERA FROM CHICKENS INFECTED FOR	CELLS FROM CHICKEN %				
	190	105	138	69	73
6 days	640	80			
6 days		320			
		160*			
7 days	160				
	320*		160		
7 days	320			160	
7 days	40			20	1280
7 days			5120	80	
				320*	6120
7 days				320	320
8 days	160				
	80*				

\* Repeated after freezing and thawing one more time

TABLE VI

*Sensitivity of Different Chicken Cell Preparations Treated with the Same Virus at the Same Time*

CELLS FROM CHICKEN %		SERUM OF 7TH DAY INFECTION									
		10	20	40	80	160	320	640	1280	2560	5120
Sensitized	73	+++	+++	+++	+++	++	0	0	0	0	0
	43	+++	+++	+++	+++	+++	0	0	0	0	0
	38	+++	+++	+++	+++	+++	++	+	0	0	0
	37	+++	+++	+++	+++	+++	+++	+++	+++	++	0
	26	+++	+++	+++	+++	+++	+++	+++	++	0	0
Unsensitized	73	0	0	0	0	0	0	0	0	0	0
	43	0	0	0	0	0	0	0	0	0	0
	38	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0
	26	+++	+++	++	0	0	0	0	0	0	0
	92	+++	+++	+++	+++	+++	++	+	0	0	0

ens (Table V) This shows a striking difference depending on the source of the preparation This is better shown in Table VI where the tests were run simultaneously Included in this test is the effect of the

same sera on unsensitized cells from the same chickens. The cells from one of these are partially agglutinated, as are the cells of an older chicken (\*92) which had been repeatedly bled in the past. This sensitivity of untreated red cells from a chicken which has been repeatedly bled may become so great that a saline suspension of these washed cells will form a spontaneous pattern in the bottom of the tube. The de-

### TITER OF IMMUNE SERA WITH SENSITIZED CELLS

25°

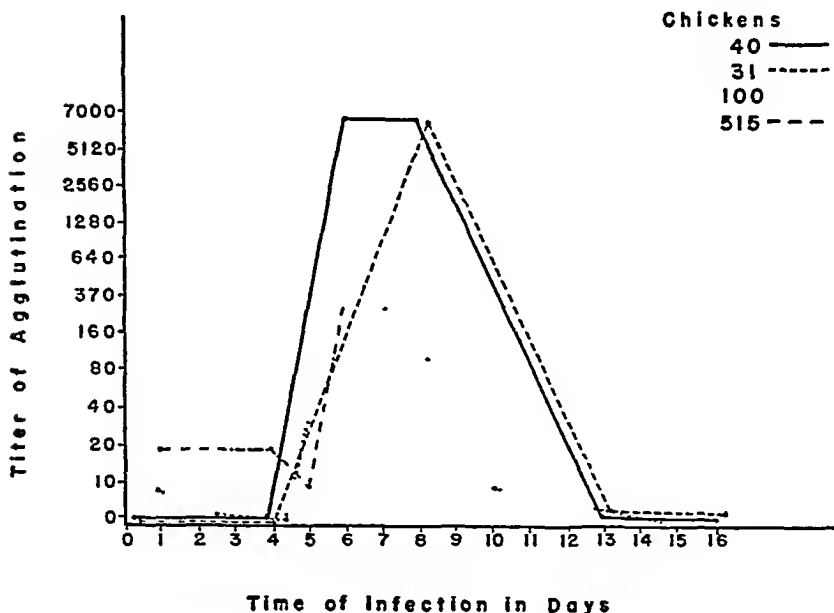


Fig 1

velopment of auto-agglutination of rabbit cells in the sera of rabbits repeatedly bled may be a somewhat similar phenomenon (7). The individual variable susceptibility of chicken cells has been noted in several other virus-red cell systems (8, 9, 10).

It was early noted (1) and confirmed (2) that when various animals including chickens were injected with the viruses of Newcastle or influenza, there developed in their serum a substance capable of agglutinating sensitized cells. It has not been determined whether

or not this virus needs to be living. However, no study of the time of its appearance and disappearance in the blood of such chickens, and no attempt to differentiate it from antibody has appeared.

As may be seen in Figs 1 and 2, the titer of this agglutinin of sensitized red cells rises sharply on about the 5th-6th day and is present particularly during the acute phase of the animal's illness. On the other hand, antibody hemagglutinin-inhibition rises perhaps a little

#### TITER OF IMMUNE SERA WITH SENSITIZED CELLS 4°

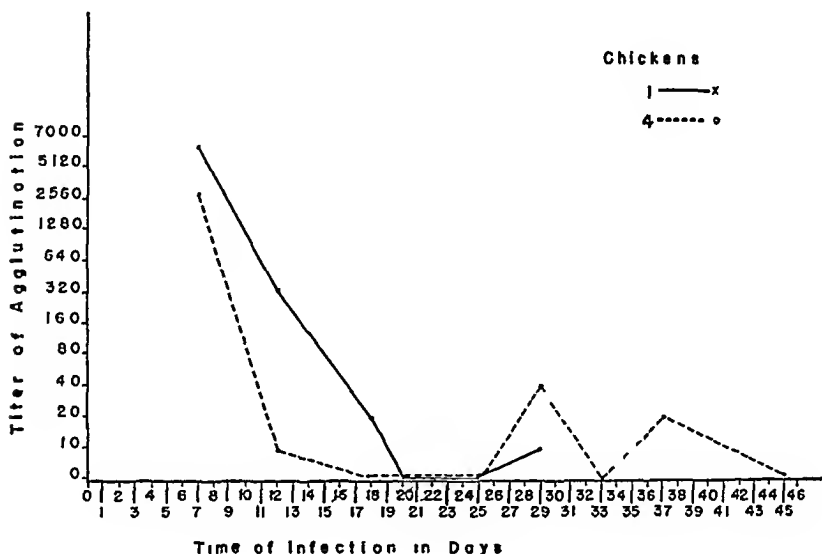


Fig 2

later and then, as is to be expected, is maintained for at least six weeks (Fig 3)

Since the agglutinin for sensitized cells is present only during the acute phase of the infection, as distinct from antibody, we attempted to determine something of its characteristics.

We found that the agglutinin obtained from acute phase sera will not dialyze through a collodion bag, that it does not precipitate when dialyzed against saline or 2 to 3 changes of water, but that it finally does come down in the precipitate with continued changes of water over a 2 day period. This is with an estimated dilution of the original

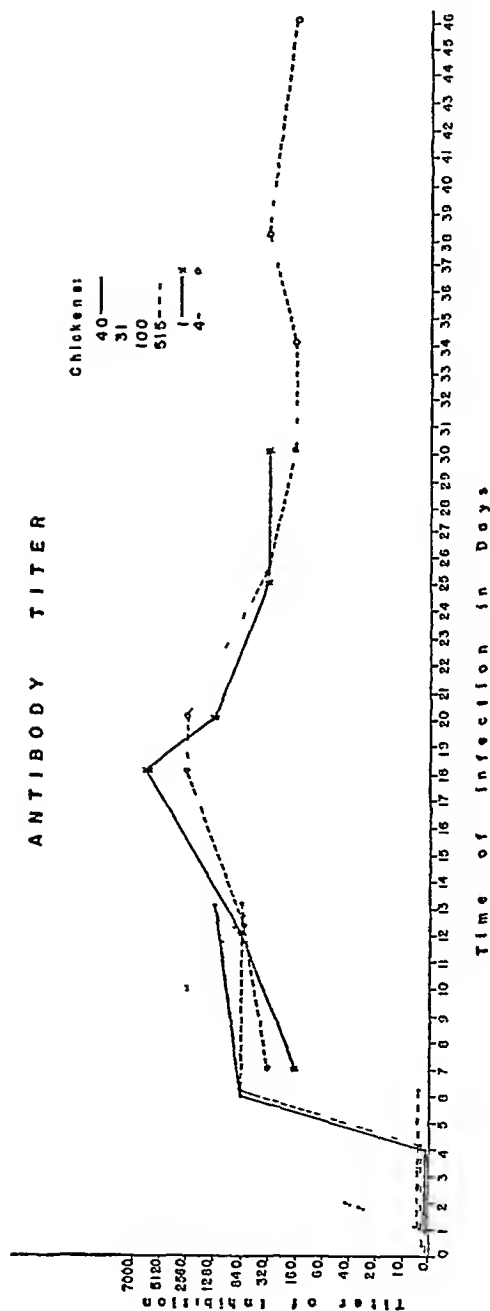


Fig 3



saline of 1/20 There was some loss of activity on dialysis which was not restored by combining precipitate and supernatant and the dialysate

It has been shown that normal chicken red cells are capable of agglutinating sensitized chicken red cells We were interested in determining wherein this factor resided and, therefore, hemolysed both normal chicken and normal human red cells It is interesting (Table VII) that human cell hemolysate produced agglutination in a 1/320 dilution whereas the ghosts produced agglutination in a 1/8 dilution of the original 1% suspension The hemolysate from chicken red cells

TABLE VII

*Agglutination of Sensitized Cells by Hemolysate and Red Cell Ghosts of Human and Chicken Origin*

HEMOLYSATE 1%	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/5120	1/10 240
Human hemoglobin	+++	+++	+++	+++	+++	+++	0	0	0	0
Fowl #1	0	0	0	0	0	0	0	0	0	0
Fowl #2	0	0	0	0	0	0	0	0	0	0
	1%	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/512	1/1024
Human ghosts	+++	+++	+++	+++	0	0	0	0	0	0
Fowl #1	+++	+	0	0	0	0	0	0	0	0
Fowl #2	+++	+++	+++	+++	0	0	0	++	++	++

failed to agglutinate any sensitized red cells in a 1/10 dilution or more, whereas one preparation of the ghosts agglutinated in a 1/8 dilution of the original 1% suspension and the other only in the original 1% It would seem then that the agglutinating substance is liberated from human red cells by hemolysis in much greater amounts than from fowl red cells This is similar to the pecten antagonist of influenza virus-agglutination extracted by Wooley from hemolysates of chicken and human red cells (11) The presence of this agglutinin of sensitized red cells in the ghosts of chicken red cells is also reminiscent of the influenza virus hemagglutinin inhibitor which has been extracted from

this same source by Hirst (12) The relationship of the agglutinin from normal red cells and that from the acute phase sera has not been studied

The presence in the blood of a proteolytic enzyme whose activity varies during the course of various diseases, including infections (13), led us to test the effect of commercial trypsin, papain, and purified trypsin\* on these sensitized cells

TABLE VIII  
*Agglutination of Sensitized Red Cells by Proleases*

	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
<i>Sensitized cells</i>										
Unpurified trypsin	0	+	+++	+++	+++	+++	+++	+	++	++
	1%	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
1% Purified trypsin	+++	+++	+++	+++	+++	+++	+++	0	0	0
Chloroform treated chicken sera	0	0	0	0	0	0	0	0	0	0
Normal sera #174	0	0	0	0	0	0	0	0	0	0
<i>Normal Cells</i>										
Unpurified trypsin	0	0	0	0	0	0	0	0	0	0
	1%	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
1% Purified trypsin	0	0	0	0	0	0	0	0	0	0
Chloroform treated chicken sera	0	0	0	0	0	0	0	0	0	0
Normal sera #174	0	0	0	0	0	0	0	0	0	0

The results of these tests are shown in Table VIII wherein it is demonstrated that sensitized cells are much more sensitive to the agglutinating action of trypsin both purified and relatively crude. Similar results were obtained with papain. This does not indicate that the agglutinin of sensitized cells is such an enzyme. Pertinent to this is the failure of chloroform treatment (14) of normal chicken serum to liberate such an agglutinin. Such treatment of human serum will liberate the proteolytic enzyme by destruction of its inhibitor. Furthermore, the effect of trypsin can be inhibited by soy bean inhibitor which has no effect on the titer of the agglutinin in the sera.

\*Courtesy Dr M Kunitz

†Courtesy of Dr O D Ratnoff

The agglutinin of sensitized red cells in chicken serum is relatively heat stable for no loss of activity was demonstrated after heating at 56°C for one half hour, but activity began to decrease after two hours exposure to this temperature (Table IX)

One of the first enzyme inhibitors tested was cyanide, at concentrations varying from 0.005 M to 0.05 M. An effect was consistently present until the concentration of the phosphate buffer in the saline was increased whereupon it disappeared, even when 0.05 M CN was used. Furthermore, the original cyanide effect was not reversed by 0.5% methylene blue. The effect of variation in the pH of the medium

TABLE IX  
*The Effect of Heating at 56°C Upon Agglutinin of Sensitized Cells*

EXP		DILUTION OF SERA									
		1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/5120	1/10 240
1	½ hrs										
	Heated normal sera	0	0	0	0	0	0	0	0	0	0
	Unheated norm sera	+	+	+	+	0	0	0	0	0	0
	Heated immune sera	+++	+++	+++	+++	++	+	0	0	0	0
2	2 hrs										
	Unheated immune sera	+++	+++	+++	+++	+++	+	0	0	0	0
	Heated normal sera	+++	+	0	0	0	0	0	0	0	0
	Unheated norm sera	+++	+++	0	0	0	0	0	0	0	0
	Heated immune sera	+++	+++	+++	++	+	0	0	0	0	0
	Unheated immune sera	+++	+++	+++	+++	+++	++	+	0	0	0

was then studied in the range pH 5.0 to 8.9. A slightly greater effect of alkaline concentrations on the sensitized reaction is apparent.

Choline esterase is mentioned by some as a participant in the reaction of influenza virus with red cells (15). Di-iso fluoro phosphate, a specific inhibitor of this enzyme, had no effect on the agglutinin titer.

Carbon monoxide (commercial gas bubbled through the saline before the addition of the sensitized red cells) and methylene blue in a concentration of 0.5% had no effect on the reaction. 0.05 M hydroquinone did not change the reaction, whereas 0.05 M sodium azide lowered it slightly. This, like the cyanide reaction, may have been due to a higher pH.

TABLE X  
Effect of Variation in pH on the Hemagglutination Reaction

INHIBITION OF VIRUS		DILUTION OF SERA										
		pH	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Hemagglutination by Immune Sera	5 04	++	+	0	0	0	0	0	0	0	0	0
	5 80	+	+	0	0	++	++	+++	+++	+++	+++	+++
	5 90	0	0	0	+	++	+++	+++	+++	+++	+++	+++
	6 30	0	0	0	0	++	+++	+++	+++	+++	+++	+++
	6 99	++	+	0	0	0	+	+++	+++	+++	+++	+++
	7 51	0	0	0	0	0	+++	+++	+++	+++	+++	++
	7 72	0	0	0	0	++	+++	+++	+++	+++	+++	++
	8 31	0	0	0	0	0	0	0	0	0	0	0
	8 95	+++	0	0	+	++	+++	+++	+++	+++	+++	++
Agglutination of Sensitized Cells by sera	5 04	+++	+++	+++	+++	+	+	0	0	0	0	0
	5 80	+++	+++	+++	+++	+++	+	0	0	0	0	0
	5 90	+++	+++	+++	+++	+++	+++	+++	++	+	0	0
	6 30	+++	+++	+++	+++	+++	+++	+++	++	0	0	0
	6 99	+++	+++	+++	+++	+++	+++	+	+	0	0	0
	7 51	+++	+++	+++	+++	+++	+++	++	0	0	0	0
	7 72	+++	+++	+++	+++	+++	+++	0	0	0	0	0
	8 31	+++	+++	+++	+++	+++	0	0	0	0	0	0
	8 95	+++	+++	+++	+++	++	0	0	0	0	0	0
Agglutination of Normal Cells by sera	5 04	+++	++	0	0	0	0	0	0	0	0	0
	5 80	0	0	0	0	0	0	0	0	0	0	0
	5 90	+	0	0	0	0	0	0	0	0	0	0
	6 30	0	0	0	0	0	0	0	0	0	0	0
	6 99	0	++	0	0	0	0	0	0	0	0	0
	7 51	++	0	0	0	0	0	0	0	0	0	0
	7 72	++	0	0	0	0	0	0	0	0	0	0
	8 31	+	0	0	0	0	0	0	0	0	0	0
	8 95	++	+	0	0	0	0	0	0	0	0	0
Agglutination of Red cells by Virus Alone	5 04	++	0	0	0	0	0	0	0	0	0	0
	5 80	0	0	0	0	0	0	0	0	0	0	0
	5 90	0	0	0	++	+++	+++	++	0	0	0	0
	6 30	+++	+++	+++	+++	+++	+++	+++	++	0	0	0
	6 99	+++	+++	+++	+++	+++	+++	++	0	0	0	0
	7 51	+++	+++	+++	+++	+++	+++	++	0	0	0	0
	7 72	+++	+++	+++	+++	+++	++	0	0	0	0	0
	8 31	+++	+++	+++	+++	+++	+++	++	0	0	0	0
	8 95	+++	+++	+++	+++	+++	+++	++	0	0	0	0

## DISCUSSION

The phenomenon of agglutination of red cells by certain viruses is in itself a complex mechanism. The problem has been extended by the discovery that certain cells, following exposure to virus, become sensitive to agglutination by certain sera and extracts of red cells. For the purpose of thinking about the action of Newcastle Disease

virus on red cells we have considered the problem in several stages. The phenomenon of agglutination here is basically one of making the red cells sticky. Unlike cells exposed to the virus of influenza, red cells exposed to the virus of Newcastle Disease do not settle more rapidly than normal cells. Even with preparations of allantoic fluid containing Newcastle Virus titring to 5,000 by hemagglutination this is true. Furthermore, as indicated at the beginning of the paper, actual adsorption of virus on the cell even with very active preparations may be minimal. Therefore, adsorption of virus of red cells, and agglutination of those red cells as they settle to the bottom of the tube, may well be independent factors. It may also be that the elution of virus from the red cells, and the sensitizing action on the red cell, are separate factors.

For these reasons we felt that the demonstration that sera taken from chickens infected with Newcastle disease allowed a more extensive analysis of the phenomenon than would be possible by studying a series of patients. Furthermore, the incomplete characterization of the serum component and its action at least allows certain things to be excluded.

It should, however, be emphasized that we have as yet no knowledge of whether the reaction between infectious mononucleosis sera and human red cells sensitized to Newcastle virus is fundamentally the same as the one here studied or not. The presence of this factor in acute sera with its disappearance in convalescent sera tends to rule out ordinary antibody (though various types of incomplete antibodies are not ruled out). Its heat stability and preservation in the icebox eliminates complement. The occasionally marked difference in sensitivity of red cells from different chickens raises the question of the role of antigens uncovered by the process of sensitization and differing from one chicken to another. Finally, the repetition of the phenomenon with certain parts of the hemolyzed human and chicken red cell turns one's thoughts towards inhibitor-like substances.

#### SUMMARY

Chicken red cells "sensitized" by exposure to Newcastle virus may be agglutinated by sera and red cell hemolysates which do not agglutinate normal red cells. Four approaches have been used in trying

to understand this phenomenon. The effects of (1) different temperatures, (2) different strains of virus, (3) cells from different chickens, and (4) the occurrence and stability of the agglutinating sera.

Sera taken from chickens five to seven days after they are inoculated with avirulent Newcastle virus will agglutinate "sensitized" cells, but fail to agglutinate normal cells. This characteristic of the sera does not obtain during the subsequent few days of the chicken's convalescence, antibodies (anti-virus hemagglutinins) having meanwhile appeared and persisted.

The factor in the serum responsible for the agglutination of sensitized cells is resistant to a temperature of 56°C for one hour, it does not dialyze through a collodion bag, it is preserved for 13 months in a frozen state, and is partially precipitated by dialysis against distilled water.

Sensitized cells were agglutinated by a hemolysate of human red cells and by the washed ghosts of normal chicken cells.

Attempts to characterize the factor by some of the usual enzyme inhibitors failed. Proteases, such as purified and crude trypsin and crude commercial papain agglutinated sensitized chicken cells and not normal cells in the concentrations used. This agglutination was inhibited by soybean inhibitor. However, chloroform treatment of normal sera which in mammalian sera releases the fibrinolytic enzyme, failed to produce an agglutinin of sensitized cells, and soybean inhibitor of trypsin did not inhibit agglutination by the positive chicken sera.

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# SPINAL SUBDURAL ABSCESS DUE TO A CONGENITAL DERMAL SINUS AND ACCOMPANYING CHANGES IN THE AUTONOMIC NERVOUS SYSTEM\*

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The following case of a congenital dermal sinus with an accompanying spinal subdural abscess has all the characteristic sensory and motor changes that Walker and Bucy included under their clinico-pathological entity (1) The case ran essentially the same course as did those described by Walker and Bucy, by List (2), and by Freedman and Alpers (3) In addition this case presents wide-spread and long lasting autonomic effects, objective records of which were obtained by means of the electrical skin resistance method

Previous papers give a full description of the electrical skin resistance mapping method and of the underlying principles (4) It will suffice to state here that the electrical resistance of the skin varies inversely with the activity of the sympathetic nervous system a high skin resistance indicating little or no sympathetic activity, a low resistance indicating marked sympathetic activity The dermatometer used for the test consisted of either a  $4\frac{1}{2}$  or  $22\frac{1}{2}$  volt battery, a 1000 ohm potential divider, and a 50 microammeter One electrode was attached to an ear lobe by means of a small silver plated nickel clasp, the other, an exploring electrode, consisting of a silver plated disc or solid metal roller attached to a wooden handle, was held in the hand of the examiner and so could be moved from place to place on the skin

This dermatometer was used only to define the boundaries of areas with various levels of increased or decreased sympathetic activity, not to obtain an actual measurement of skin resistance in ohms

\*Carried out under a contract between the Office of the Surgeon General of the U S Army and The Johns Hopkins University

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*D L E First admission June 14 to August 17, 1944*

This one year old girl was admitted with paralysis of both legs. The illness had started when she was 2 weeks old and a small draining sinus was noticed in the midline of the lower back. The fluid that drained from the sinus was at first clear, but after 6 months changed to pus. Three months later the sinus was excised. Following operation the operative site drained profusely for a short time but healed completely by the end of 6 weeks. The child remained well for several weeks, then became weak in the legs. This condition progressed until 2 weeks before admission when both legs became totally paralyzed.

On admission the temperature was 103.6° and the child was restless and uncooperative. The abdomen was markedly distended with a huge bladder. Catheterization yielded 460 cc of urine. In the lower lumbar region in the midline an indurated area was present and the tiny opening in its center was not draining. Horner's syndrome was present on the right side. There was a loss of sensation on the right side from the nipple down, and on the left from the inguinal region down. The knee and ankle jerks were absent on both sides.

Laminectomy performed by Dr F Otenasek revealed the presence of a huge intradural abscess. This was opened and drained. The child improved immediately, profuse drainage present for a few days quickly receded. Recurrent bladder infection and constipation constituted the main post-operative difficulties. An indwelling catheter was required until August 8. After this she had to be catheterized 3 times daily. Daily doses of mineral oil relieved the constipation. The infection was treated with penicillin and sulfadiazine.

By the time of discharge the child was able to walk when aided and the urinary infection had cleared up.

*Interval Note* October, 1944. Two months after discharge the child was seen again. She had improved steadily. Walking with slight support was possible. The right side of the body from the head to the umbilicus did not perspire. The child still had no bladder control. The abdominal reflexes on the right were diminished. The tendon reflexes in the right leg were still absent.

*Second Admission* November 6-27, 1947. The child was not seen

again until November 1947, when she was admitted to the Johns Hopkins Hospital for the second time at the request of her local physician

Since last seen, the child had made remarkable recovery. She had grown normally, and had had no illnesses of any kind except for recurrent bladder infections. Since the age of 2 years she had been walking alone. The child was alert, active, and bright. She was still incontinent of urine. Her mother catheterized her three times daily. Constipation remained a problem. The lumbar sinus continued to exude a few drops of pus daily.

*Physical Examination* Temperature, pulse and respiration were normal. A long midline scar was present over the lumbar spine. In the region of L<sub>4</sub> there was a small mound of granulation tissue with a sinus in the center from which a drop of pus could be expressed. The right leg appeared to be about 2 cm. shorter than the left. On standing, there was a scoliosis with an upward tilt of the pelvis on the right.

Neurological examination showed no discrepancy in the cranial nerves. The Horner's syndrome was no longer present. Muscle strength, tone and movement were unimpaired in the arms. In the legs, however, there was marked hypotonus on the right. The muscles were not as well developed as on the left, the right thigh being 2 cm. less, and the calf being 1½ cm. less in circumference than the corresponding parts on the left. Strength in all muscle groups was less than on the left and the joints were relaxed. The right ankle was unstable and dorsiflexion poor. The toes could not be moved voluntarily. On the left, strength and tone were much better but there was definite weakness of dorsiflexion and plantar flexion of the foot.

The child was cooperative and accurate on sensory examination. Touch was intact, as were deep pressure and position senses. Vibration was lost at the ankles but appreciated at the knees. Pain sensibility was diminished on the right everywhere below the umbilicus, it appeared to be lost on the posterior aspect of leg and over the buttock. On the left pain appreciation was diminished over S<sub>1</sub> and absent over the buttock. There were no cerebellar difficulties. The tendon reflexes in the arms were intact. The abdominal reflexes on the right could not be obtained. The right knee kick was diminished and the right

ankle jerk was absent. The corresponding reflexes on the left were normal. No pathological reflexes were obtained.

There was a limp on walking and the right leg was used as a prop. The right ankle tended to evert. The leg was strong, however, and would bear her weight. The back was limber and could be bent in all directions without discomfort.

Leucocyte count showed 18,000 cells per cu mm. X-rays of the lumbar regions showed that the spinous processes of the 3rd and 4th lumbar spines were missing. There was no evidence of bone destruction or of osteomyelitis.

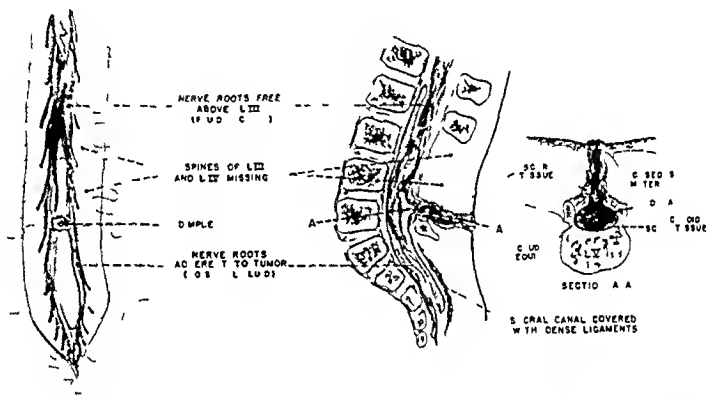


FIG 1 Drawing made at the operating table showing the sinus within the dural space

The sinus was injected with thorotrast and the opaque material filled the sacral portion of the spinal canal. On November 12, 1947, the old laminectomy incision was opened. The sinus tract was excised as a core, and followed down into the spinal canal. It ran through the dura, where it was connected with a long sausage-shaped mass, which extended from  $L_1$  to the coccyx. (See drawing in Fig 1). The roots were adherent to the outside of the mass by dense arachnoid tissue, but were not incorporated in it. In order to expose the tumor it was necessary to remove the lamina at  $L_{1-2-5}$  ( $L_{3-4}$  having been previously removed). The sacral canal was covered with ligamentous tissue only. It was possible to remove the tumor completely without

injury to the roots except for a few of the lower sacral filaments. Pathological study of the mass showed that it was a cystic tumor lined with stratified squamous epithelium.

The post-operative course was uneventful. The wound healed per primum without evidence of infection. It was necessary to catheterize the child for several days. She was able to walk with support on the 8th post-operative day and by the date of discharge on November 27, 1947 she could once more walk alone. The neurological status was unchanged. Cystometric study of the bladder showed increased capacity but good tone and normal tracing. It was noted that there was no pain or desire to void when the bladder was distended.

*Interim Notes* January 26, 1948. The patient walks well but with a limp, using the right leg as a prop. She still is unable to move the toes of the right foot and has only slight control of movements of the right ankle. Feels pain as far down as S<sub>1</sub> on the right; no anesthesia on the left. Has better control of bladder and bowel.

*June 7, 1948* Patient has continued to improve. The knee and ankle jerks are still absent on the right side. She has some voluntary control of movements on right foot. The right leg is 1 cm. shorter than the left.

*April 20, 1949* In the standing position the patient's right foot is slightly everted. She walks well but with a limp. Her right knee and ankle jerks are still absent. She is able to extend the small toes of the right foot but has no power in the extensor hallucis longus or the anterior tibial muscle groups. The right peroneal muscles are quite strong. The hamstring muscles of the right leg are approximately 50% of normal strength. Plantar flexion of the right ankle is approximately 30% of normal strength. The patient has developed considerable control of her urinary bladder and rectal sphincter.

#### *Electrical Skin Resistance Examination*

This patient was examined 8 times over a period of 5 years beginning with an examination on the day of admission. Fig. 2 shows the skin resistance patterns that were found on three different occasions.

*June 14, 1944* This examination made on the day of admission revealed a sharply defined unilateral area of increased skin resistance (decreased sympathetic activity) that included the entire right half

of the body. A line at  $T_9$  sharply divided an upper area with a very high resistance (solid black) from a lower area with only an intermediately high resistance (cross hatching). Above this line the resistance was as high as is found after a complete sympathectomy.

*June 7, 1948* This examination made 4 years after the initial admission revealed a sharply defined unilateral area of increased resistance that still included the entire right side of the body (except for small facial area). The magnitude of the increase in resistance was much smaller than that present in 1944.

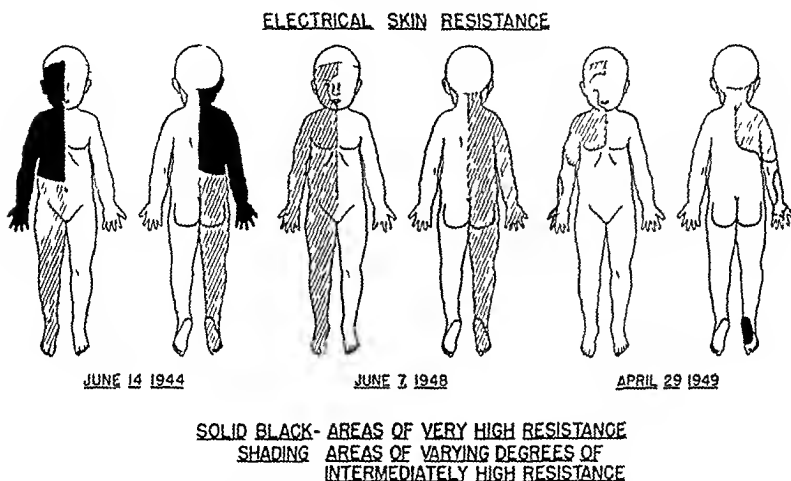


FIG 2 Body charts showing the areas of high, intermediately high and normal electrical skin resistance at various stages after the development of the subdural sinus.

*April 27, 1949* The examination showed that the area of increased resistance had diminished since the last examination. It included the head (except for facial area), neck, shoulders, part of the arms, and the trunk down to  $T_5$ . The increase in resistance was slight but still very definite. To ordinary visual and tactual inspection, however, sweating appeared to be equal on the two sides.

Increased resistance was not found on the trunk and legs, except for an area (solid black) on the plantar surface of the foot. The resistance level of this area was as high as is found after a complete section of a peripheral nerve. In the previous examinations its presence probably was missed.

The electrical skin resistance examinations have shown that the effects produced by the subdural abscess on the sympathetic nervous system may be strictly unilateral. No deviation from normal levels of resistance were observed at any time on the left side of the body, in spite of the presence on that side of definite alteration in sensory and motor functions. The effects produced on the sympathetic nervous system are, in this case, long lasting, being present even after 5 years, however in greatly diminished intensity. They persisted longest in the head and upper thoracic region, the parts of the body farthest removed from the site of the sinus. It is noteworthy that these changes were present in the absence of any abnormalities of the sensory and motor system in this part of the body. The plantar surface of the foot showed an area with an apparently permanent sympathetic denervation.

#### SUMMARY

This patient was born with an intradural dermal sinus in the lower lumbar and sacral parts of the spinal canal. It drained freely through an opening between the 4th and 5th lumbar vertebrae. At the age of 4 months this draining sinus became infected and at 7 months the excision of the extradural part occluded the sinus and blocked the drainage from the intradural part. The resulting increase of pressure must have first caused the sinus to become very much distended and later may have caused it to rupture, allowing the enclosed pus to spread freely through the subdural space. Pressure from the abscess and/or local inflammatory action on the spinal roots, produced widespread asymmetrical changes in the motor, sensory and autonomic nervous systems. Below T<sub>11-12</sub> the effects were bilateral, above strictly unilateral.

At the end of the first year drainage of the sinus and treatment with penicillin brought almost immediate relief. The child quickly became able to walk and to carry on many activities. Her bladder control still remained poor. Two years later when all the infection had subsided the intradural part of the sinus was readily removed but some of the sacral roots may have been injured in the process. The child made a rapid recovery. Some sympathetic motor and sensory changes are however still present but mainly on the right side in this now 5 year old child.

## DISCUSSION

An attempt has been made to reconstruct the series of events that led to the changes in the motor, sensory and sympathetic nerves. When the child was first seen the paraplegia and sensory levels ( $T_4$  on right,  $T_{11}$  on left) may have been produced by pressure of the distended infected sinus and the subdural abscess on the spinal roots and cauda equina. Some of the damage may also have resulted from direct inflammatory changes in the roots.

The fact that the sensory and sympathetic changes were asymmetrical on the two sides of the body is somewhat disturbing. At one time the loss of sensation extended on the right to  $T_4$ , the sympathetic paralysis up to  $T_1$ , while similar changes were never higher than  $T_{11}$  on the left. It is extremely unlikely that the intradural portion of the dermal sinus extended up the right side of the canal to the upper thoracic region. A subdural abscess however is not restricted in its spread, and could easily have reached that level. It is also not difficult to visualize that it spread higher on one side of the cord than the other. While the infection may have spread that high, the neurological deficits may not have been due to pressure by pus, but may have resulted from an inflammatory reaction. This latter possibility is suggested in view of the slow regression of the sympathetic changes, since alterations in skin resistance were noted in the head and arm long after any pressure from an abscess had been relieved. It might be mentioned that at no time was there evidence for a subarachnoid extension of the infection or for a true myelitis.

Why the sympathetic fibers were so severely injured in the head and arm area, without signs of motor or sensory loss, is not clear. The presence of permanent motor and sympathetic effects in the right foot and saddle region demonstrates that the cauda below  $L_4$  on the right underwent irreversible changes. This may have been due to prolonged pressure from the sinus and abscess, direct infection, or the subsequent arachnoiditis as remarked above. When the intradural portion of the sinus was removed the lumbar and upper sacral roots were not damaged.

Since lesions to nerves at  $L_3$  or below do not produce changes in skin resistance because the sympathetic outflow is complete at  $L_2$ , the

changes noted in the right leg must have resulted from injury to the roots at  $L_1$  or  $L_2$ . While the roots did not appear abnormal at operation, they could easily have been more or less severely damaged at the time of the acute infection. Here the damage was not so selective, and motor deficits as well as damage to the sympathetic fibers, may have resulted.

As was found in other reported cases, there was remarkable recovery after the infection was brought under control and the sinus removed.

#### SUMMARY

The history of a patient with congenital intradural dermal sinus is reviewed. During infancy the sinus became infected. Later a partial excision blocked its drainage and led to the formation of a subdural abscess. This was cured by surgical drainage and chemotherapy. At a later date the dermal sinus was completely removed.

Unilateral widespread changes in the sympathetic nervous system were noted during the time of the subdural empyema. These changes were persistent as is evidenced by electrical skin resistance readings taken as long as 4 years after the cure of the abscess.

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## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

*Tom Cullen of Baltimore* By JUDITH ROBINSON 435 pp \$3 50 *Oxford University Press, New York*

Tom Cullen of Baltimore, Oxford Press, is the biography of the only remaining member of the medical faculty whose professional life span coincides with that of The Johns Hopkins Hospital and Medical School. The eldest son of a Wesleyan minister in the bush country of the newly settled Ontario, Tom Cullen's childhood would seem rugged to the modern child but it was rich in the adventures of boys in a country well suited for the development of self-reliant men. His early education was in the public schools of the smaller communities to which his father was assigned and later in Toronto. Although encouraged at home in his efforts for an education he was forced to support himself from an early age. Even while attending medical school in Toronto his earnings from his paper route and other tasks paid his tuition and other expenses. In reading of his early life it is apparent that the tremendous drive and capacity for work which characterized his later years began at a very early age. His habit of early rising to work before breakfast, although modified gradually with passing years, remained with him throughout life.

The dynamic push which was one of Dr. Cullen's most characteristic qualities did not exclude play. He received a tremendous amount of pleasure from his work and enjoyed a little horseplay at the operating table as much as his younger assistants. In addition he was an ardent advocate of prolonged summer vacations and one of his commonest admonitions to young men was, "Remember you can do more work in ten months than in twelve." As soon as his financial position made it possible he spent two full months at his camp on Lake Ahmic in the Magnetawan country of Ontario. But on returning in September his drive for work was unremitting until the following July.

In 1891 young Tom Cullen came to Baltimore as an interne under Dr. Kelly. From that moment on, his "chief" became his hero and remained so until Kelly's death. With almost dog-like faithfulness he carried out the "chief's" wishes even when he had to swallow hard to accept some of Kelly's views. After completing his training as a gynecologist Cullen's professional life was spent in Baltimore and eventually he succeeded his master as Chief Gynecologist of The Johns Hopkins Hospital and as Professor of Gynecology of The University. He served in this dual capacity until he reached the age of retirement in 1939. In the two decades during which Cullen occupied the Chair of Gynecology, Hopkins was torn with family feuds in which Cullen was embroiled. The biographer tells the story of the fight on full time professorships which divided the faculty sharply into two camps.

She also relates the gynecological-obstetrical feud in which Cullen and Williams played leading roles. After the passage of many years these stories are told without rancor but let the reader not be misled into believing that it did not exist at the time.

Cullen's greatest contribution to gynecology was his development of the gynecological pathology laboratory. The interest which he inspired in this phase of gynecology has had a more profound and important influence on his specialty than any of his other accomplishments. Another accomplishment of almost equal importance to medicine was the work he did single-handedly in establishing a Chair of Medical Art at the Hopkins. This chair was occupied with distinction for many years by his devoted friend, Max Brodel, whose work established art as applied to medicine in this country.

It is regrettable that the biography, which in a sense is a history of the Hopkins gynecological department does not tell more of the accomplishments of Cullen's co-workers in the development of this department.

On the occasion of his seventieth birthday Tom Cullen's friends gave a testimonial dinner to him which was attended by a host of friends and admirers. This was the end of his University life but not of his career. He continued actively in practice and made his greatest contribution to the City of Baltimore as President of the Board of the Enoch Pratt library. He was elected to this post in 1938 and his faithful performance of his duties and great enthusiasm for the cause have resulted in his re-election each year since.

The book is written in a readable style by Judith Robinson. Her style is light enough to be entertaining and factual enough to be of value as an historical document covering an important and long era in The Johns Hopkins Medical Institutions. Much of it is in direct quotation by Tom Cullen in his inimitable style. His personality almost steps out of the pages. To all Hopkins alumni, to Baltimore citizens and to Tom Cullen's many friends throughout the country the volume will prove to be highly pleasing reading.

R. W. TE LINDE

*The Diagnosis of Pancreatic Disease* By LOUIS BAUMAN. 74 pp. \$5.00. J. B. Lippincott Co. Philadelphia.

The diagnosis of disease of the pancreas is always difficult. Within the last few years a number of studies have appeared in which the enzymatic properties of pancreatic juice have been analyzed as a diagnostic aid. Dr. Bauman, in this monograph, summarizes his experience in the laboratory diagnosis of pancreatic disease. Briefly, Bauman's technique consisted of passing a double-barrelled tube into the duodenum. Through one half of the tube, the pancreatic juice was aspirated. Through the other half, the gastric juice was neutralized by calcium carbonate. The duodenal juice was collected in 10 minute fractions into iced receiving tubes, and analyzed for its content of amylase, protease, and lipase. Mecholyl was used as a stimulant of pancreatic secretion because secretin, as expensive, and tended to be toxic.

Dr Bauman does not believe that one should aspirate the pancreatic juice to assist in the diagnosis of acute pancreatitis, although others have reported a deficiency in amylase and protease in this disease. In chronic pancreatitis and carcinoma of the head of the pancreas, Bauman observed that the concentration of pancreatic enzymes was decreased. However, in cancer of the body of the pancreas, he found that the pancreatic enzymes were usually normal.

In interpreting Dr Bauman's data, one must bear in mind that the great variations in enzymatic activity are observed in patients who apparently do not have pancreatic disease. Moreover, the data presented indicate that mecholyl is a poor stimulant of the flow of lipase in most patients. In his discussion of sprue, Dr Bauman confirms the fact that the pancreatic secretions in this disease are usually normal. He does not remark that half of his patients had a fasting level of lipase which was less than the minimum normal value. The significance of this observation is not clear.

This small volume is well-written and is published in the most attractive way. However, it seems unnecessary to charge \$5.00 for a book which is 74 pages in length. Undoubtedly the large amount of tabular material which is presented is difficult to set. However, a less expensive format and binding, might have permitted making this valuable little book more attractively priced and therefore more accessible to the average medical reader.

OSCAR D. RATNOFF, M.D.

*Physiology in Diseases of the Heart and Lungs* M. D. ALTSCHULE 368 + XV pp  
\$5.00 Cambridge, Harvard University Press, 1949

The author, who has contributed many precise measurements, analyses and speculations to the field embraced by his book, has now written a monograph which is difficult to classify. In the preface there is outlined the goal of providing the student (pre- or postdoctoral) with source material derived from laboratory and clinical research so that, "The student may be stimulated to look into this book for the explanation of an unusual or, to him, unexpected clinical phenomenon, or he may merely browse through it when he feels no urge to do anything else." There can be no cavil at this aim which responds to an urgent need.

In order to provide this material the author has written a series of brief essays within the framework of more inclusive disease patterns. For example, the first section on *chronic cardiac decompensation* occupies 217 pages consisting of 37 essays on such topics as cardiac output, venous pressures, respiratory dynamics, extracellular fluid volume, renal function, skeletal muscle function, cyanosis, etc. The following 85 pages are devoted to other patterns of cardiac disease. The last 52 pages are devoted to several representative diseases of the respiratory apparatus.

The style of these brief essays is determined by the author's decision to provide almost encyclopedic references to the vast body of observations recorded, for the most part, in the past 40 years. Thus, an average page is peppered with 20 or more references. This does not make easy reading. Each brief section is followed by a bibliography often occupying four to five pages.

The enormous value of this careful work is the provision of what is virtually an annotated bibliography with sensible, critical evaluation. For the thoughtful student, many bare statements may be too elliptical to be satisfying. Perhaps this will stimulate reference to the original reports which are listed in such generous profusion. If this be the case, one goal will have been reached.

It is probably a reflection of the fluidity of the subject that appears in these essays as an unwelcome similarity to some of the synoptic reviews of recent years. Despite these lesser shortcomings, this book is recommended without reservation to all physicians and biologists concerned in any fashion with problems of circulation and respiration.

J L L, JR

*Medical Clinics on Bone Diseases, 2nd edition* By I SNAPPER 292 pp \$20.00  
Interscience Publishers, Inc New York 3, N Y

This profusely illustrated and documented atlas and text emphasizes the metabolic aspects of the etiology of bone diseases. It follows that knowledge concerning the changes brought about by hyperparathyroid disease and by hyperplasia of the parathyroids secondary to kidney disease are given great prominence. Bone changes secondary to multiple myeloma, to various anemias, Gaucher's disease are considered in detail. Illustrations of osteosclerotic and osteoclastic responses in Hodgkin's are striking. A valuable feature is the case reports, some from the literature, many from the author's own experience. A good book such as this might be even more valuable had it included some reference to tumors of the bone, osteomyelitis and syphilis. However, the author does not pretend to complete coverage.

Taxonomists of insects are frequently listed as "splitters" or "lumpers" in their consideration of the specific and generic relations of the various species. Dr Snapper is apparently a "lumper" when it comes to the varieties of lesions described in bone diseases. This, no doubt, is good when dealing with a poorly understood group. One wishes, however, that eponyms had been used at an absolute minimum. Actually we frequently find some worthy investigator's name attached to the cells, the symptoms or signs and the disease complex.

This is, however, a good and worthwhile book, which will serve as a frequent reference in any large medical clinic.

F B B

*A Textbook of Neuropathology* By BEN W LICHTENSTEIN, BS, MS, MD  
Published by W B Saunders Company, Philadelphia and London, 1949  
pp 474 \$9.50

As the author mentions in his preface, this new book has been prepared primarily for the medical student. He introduces the reader to alterations in structure of the nervous system in a satisfactory general fashion. Tissue changes are discussed primarily in terms of possible causative factors rather than as discrete disease entities. Of necessity, this form of presentation leads to rather artificial

grouping in some instances. Controversial subjects are minimized. The author is found adding many short inadequate descriptions of far too many rare, or not so rare, entities which don't quite fit in. Thumbnail sketches of clinical topics keep appearing in a fashion which might lead to confusion. These adornments, like the brief chapter on neuroanatomy seem far too inadequate and might well have been omitted.

It is unfortunate that these features tend to detract from the fundamental value of this book. The general plan and treatment is good, and much needed. The writing is clear and interesting. The illustrations are, in most cases, excellent.

J W M

*The Practice of Refraction* By SIR STEWART DUKE-ELDER 5th Edition Illus 290 pp \$6.25 *The C V Mosby Company, St Louis, Missouri*, 1949

This new edition has been thoroughly revised and brought up to date while preserving the size of the previous editions. The original lucid style used in describing the principles of refraction has been maintained.

Data on the variability of the axial length of the eyeball, as determined roentgenologically, has been added. The section on anomalies of refraction has been expanded to include a more thorough description of aniseikonia and of transient changes in refraction. New material also has been added to the sections dealing with the accommodation-convergence mechanism, with heterophoria and with contact lenses.

Sir Stewart Duke-Elder's book well deserves its popularity and certainly will maintain its position as one of the foremost textbooks on refraction.

W G M

*Care of the Surgical Patient* By JACOB FINE *W B Saunders Co, Philadelphia*, 1949 544 pages \$8.00

There is a great deal of wisdom and much sound advice in this lucid and comprehensive book. The style and the treatment are rapid and conversational. An astonishing amount of ground is covered. There are good sections on fluid balance and nutrition, sections covering a great diversity of conditions in all the special fields and types of surgery, sections on laboratory methods and special sections on the general aspects of preoperative and postoperative care. The treatment of most sections is of necessity didactic and brief, references are few and no bibliography is given. The book will be most useful to the student or practitioner interested in a rapid review such as might be given in a lecture series.

M M R

*Male and Female* By MARGARET MEAD 477 pp \$5.00 *William Morrow & Co New York* 16

Margaret Mead believes that the quality of our civilization is threatened, because we falsely delimit maleness and femaleness, and that human culture will

progress or decay only so far as we use the gifts and respect the inherent differences peculiar to each sex

One threat to our full cultural growth is the tendency to limit certain professional, intellectual and familial practices to one sex and as a corollary to impose a social penalty on the other sex for participation in that practice Dr Mead examines fully, and fairly, the physical and the learned differences between male and female and pleads for careful judgment in whatever changes we make in recreating the tasks of both as we build for the future The American marriage ideal is almost impossibly difficult to maintain, based as it is on romantic love, financial security and spiritual fidelity, and it can achieve stability only by complete mutual respect for the mind, body, and ideals of the co-partner

Anthropology is the logical discipline from whence a warning to pause and take stock of our social development should come Dr Mead analyzes the swift changes in our culture against a background of seven South Sea cultures whose biological equipment and basic heritage are the same as ours Sexual intercourse is immensely enjoyed by the Samoans, detested by the cannibal Mundugumor of New Guinea, tolerated as an economic necessity by other peoples, and set in a graceful ritual by the Balinese And, just as the sex act itself is affected by customs locally imposed, so is the way of life of the people a reflection of their attitude toward the role of male and female

In our country the pattern of contemporary living is affecting the basic biological cycle Wars have left a surplus of women, the rearing of children and the life of the family has become a matter of keeping up with the Joneses in double harness, while observing obscurely defined taboos in business and in intellectual pursuits

One world, unified by technology, and in a gradually levelling intellectual environment, is on the horizon Dr Mead would have us stop, while we can still alter the blueprints, and think on these things

B G B

*Medical Etymology* By O H PERRY PEPPER 263 pp \$5 00 *W B Saunders Co*, Philadelphia

This interesting attempt at furthering one phase of medical education and easing the burden of the medical student will meet a varied reception It is a welcome guide to the meaning—from the etymological point of view—of medical words Despite the necessary interest in eponyms, one welcomes the author's statements that "eponymic titles are given to diseases only for lack of something better eponyms persist and defy the advance of knowledge, but must always lose out in the end, when science learns enough to justify the coming of some appropriate term Often an eponym is a clear indication of our ignorance and constitutes a challenge to the investigator to rid our terminology of one more eponym"

The book is in no sense a dictionary No attempt is made even to tell you that *Aedes* and *Anopheles* are mosquitoes—only that *Aedes* means unpleasant and

Anopheles harmful, and furthermore to discover this, the reader must know that he should look under the parasitology section

There are errors and inadequacies. For instance, the statement "*variolation*, meaning to vaccinate with cowpox virus" and under *Giardia* the inference that this is a worm. The entire definition of trophozoite as "a stage of the malarial parasite" would be misleading to the student, who was at the time reading about amoebiasis.

Despite such scattered inconsistencies, this is a good book and a worthwhile attempt.

F B B

## BOOKS RECEIVED FOR REVIEW

- A Year with Osler* By JOSEPH H PRATT 209 pp \$4 00 *The Johns Hopkins Press, Baltimore, Md*
- Atlas of Obstetric Technique, 2nd edition* By PAUL TITUS 197 pp \$7 50 *The C V Mosby Co, St Louis, Missouri*
- Tom Cullen of Baltimore* By JUDITH ROBINSON 435 pp \$3 50 *Oxford University Press, N Y*
- Fundamentals of Otolaryngology* By LAWRENCE R BOIES 443 pp \$6 50 *W B Saunders Co, Philadelphia, Pa*
- Hemoglobin* By F J W ROUGHTON AND J C KENDREW 317 pp \$8 50 *Interscience Publishers, N Y 3*
- Histopathology of the Skin* By WALTER F LEVER 449 pp \$10 00 *J B Lippincott Co, Philadelphia, Pa*
- Industrial Hygiene and Toxicology, vol 2* By FRANK A PATTY 1138 pp \$15 00 *Interscience Publishers, N Y 3*
- Life Among the Doctors* By PAUL DE KRUIF 470 pp \$4 75 *Harcourt, Brace & Co, N Y 17*
- Male and Female* By MARGARET MEAD 477 pp \$5 00 *William Morrow & Co, N Y 16,*
- Marihuana in Latin America The Threat it Constitutes* By PABLO OSVALDO WOLFF 56 pp \$1 50 *Washington Institute of Medicine (sponsors) Linacre Press, Inc, Washington 6, D C*
- Medical Clinics on Bone Diseases, 2nd edition* By I SNAPPER 292 pp \$20 00 *Interscience Publishers, Inc, N Y 3*
- Nervous and Neurohumoral Regulation of Intestinal Motility* By W B YOUNG 129 pp \$4 75 *Interscience Publishers, Inc, N Y 3*
- Operations of General Surgery, 2nd edition* By THOMAS G ORR 890 pp \$13 50 *W B Saunders Co, Philadelphia, Pa*
- Physiology in Diseases of the Heart and Lungs* By MARK D ALTSCHULE 368 pp \$5 00 *Harvard University Press, Cambridge, Mass*





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